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A PLETHYSMOGRAPHIC STUDY OF SHOCK AND STAMMERING

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The experimental work here reported was begun in May, 1917, and continued until October, 1918. The intention at the start was to test the organic reactions accompanying stammering, with special reference to Dr. C. S. Bluemel's cerebral congestion theory of stammering, and this object has been kept in mind throughout.

As I was unable to secure as subjects any stammerers who had been trephined and as it has been shown that the changes in volume in the hand or finger represented the changes in all parts of the periphery, the only expressive process studied was the change in volume of either the right hand or the second finger of the right hand.

The introspections of all severe stammerers agree that they frequently experience, when called upon to say something of importance, a great fear that they will stammer if they attempt to speak. This fear sometimes is so intense that the stammerer's mind becomes a blank and he stands immovable and speechless; his eyes bulge from their sockets, the perspiration streams down his back, he opens his mouth and may even tremble, thus exhibiting all the symptoms of real fright. It seemed worth while, therefore, to arouse, in both normal speakers and stammerers, an emotion similar to that of fright by producing shock by such means as the unexpected blast of a shrill whistle, and to compare the reactions of normal speakers to shock with

those of stammerers to shock, and the stammerer's reactions to shock with his reactions to stammering.[1]

Before describing my experiments, it will be well to review briefly the results obtained by other investigators.

History of experimental literature. It is needless to review in detail the investigations included by Anderson (1), Angell and Thompson (3), Shepard (29), Stevens (32) or Weber (35) in their historical résumés. I will begin, therefore, with a summary of the results obtained by all the experimenters who have, to my knowledge, worked on this problem, and I will then review the results obtained by two recent writers whose work has a special bearing on my experiment.

Table 1 gives the direction of the changes in arm, brain and intestinal volume during fright, sensory stimulation, pleasant versus unpleasant emotions or sensations, tension versus relaxation, physical work and mental work, as reported by every experimenter who has to my knowledge worked on this problem, together with the dates when the experiments were performed if they are given (otherwise the date of the publication), the number of subjects used by each experimenter (where given), and the number of experiments performed by each experimenter (where given).

All five experimenters who obtained reactions to fright found that fright almost always occasioned a marked decrease in peripheral volume, with or without a temporary rise when stimulus was given; Küppers alone reported an increase in a small percentage of his experiments. Weber found an increase in intestinal volume. The three who experimented on the brain found an increase in brain volume at some point on the record; Berger found an increase followed by a decrease, Mosso an increase only, Shepard a short increase followed by a brief return to normal, then a long characteristic increase.

Fourteen experimenters found that sensory stimuli, in a very large majority of cases, brought about a decrease in arm volume less marked

[1] It is unfortunate that some American writers have employed misleading definitions for "stammer" and "stutter." Some writers use "stammer" to denote a stoppage of speech and "stutter" to denote the repetition of the first letter or syllable of certain words. Shepard and some other writers use "stutter" to denote both repetition and stoppage, and "stammer" to denote mispronunciation, as in baby talk and lisping. As stuttering usually develops into stammering and as many defects of speech consist of both as first defined, I shall use "stammer" throughout this discussion as absolutely synonymous with "stutter" as defined in Webster's *New International Dictionary*—"to make involuntary stops in uttering syllables or words."

than in fright, and preceded less often by a temporary rise; Lehmann alone reported an increase for touch stimuli and a decrease both preceded and followed by a temporary increase for visual and acoustic stimuli. All three experimenters found a rise in brain volume.

Sixteen experimenters compared the vasomotor changes in the periphery accompanying pleasant versus unpleasant emotions and sensations, and all found that the unpleasant states brought about constriction. Eleven found that the vasomotor changes were the same for pleasant as for unpleasant states; five found the opposite to be the case. As but five experimenters, whose interpretations of their results are questionable, found opposite vasomotor changes for pleasant and unpleasant states in but few experiments on but few subjects, and as eleven experimenters, including the most accurate experimenters we have, found no difference in the reactions to these states in about nine thousand experiments on a large number of subjects, the evidence is overwhelming that all emotions and stimuli, whether pleasant or unpleasant, have a very strong tendency to cause vasoconstriction in the peripheral arteries. As the work done by Shepard is absolutely trustworthy, and as he performed one hundred and eighty experiments on two trephined subjects with reliable apparatus, I think his conclusions that all emotions and sensations cause vasodilatation in the brain during the waking state, may be accepted until we have reliable additional evidence to support the opposite view.

Only three experimenters have compared the bodily reactions during a state of tension with those during a state of relaxation. These all found that tension caused vasoconstriction in the peripheral arteries, whereas relaxation caused vasodilatation.

Only two experimenters have studied the vasomotor changes during physical work. Anderson found vasomotor dilatation in the arm in 75 per cent of the reactions from subjects in good physical condition and in but 30 per cent of the reactions from subjects in poor physical condition. Weber found vasodilatation in both the arm and the brain and vasoconstriction in the intestines, when the subject was not tired, and the reverse in the arm and the intestines when the subject was fatigued. There are not sufficient data upon which to base any conclusions as to cerebral and intestinal vasomotor changes during physical work.

The results concerning changes in peripheral volume during mental work are rather contradictory. Most experimenters made no exhaustive study of this problem but requested a few subjects to solve a few

TABLE 1

EXPERIMENTER	DATE	NUMBER OF SUBJECTS	NUMBER OF EXPERIMENTS	FRIGHT	PLEASANT	UNPLEASANT	STIMULI	TENSION	RELAXATION	MENTAL WORK	PHYSICAL WORK
Arm volume											
Anderson	1915–7	30	7000		No difference					+ (−)+ 75%	+75%
Angell and McLennan	1896				+ (−)	−90%				+25% −75%	
Angell and Thompson	1899	2	"Complete"	−	−	−	−			+50% −50%	
Berger	1904–7							−		−	
Binet and Courtier	1896			−	−	−	−			±	
Binet and Henri	1895				−	−	−			−	
Bonser	1903	12			− (+)−	− (+)−				+−	
Citron										−	
Drozynski	1910				+ (−)	−					
Fere	1887				+	−					
Frankfurther and Hir.	1909						−			−	
Gent	1903	3	Few		(+) −	−	−	−	+	−	
Gley	1903									−	
Hallion and Comte	1894						−				
Kuppers	1913			− (+)	− (0)	−	− (+)			−	
Lehmann	1892–9	12	100	(+)−	(−)+	−	(+)−(+)	−	+	(+)− −	

Mosso	1879			−	−	−	−			−	
McDougall	1896		1100				−			+−	
Shepard	1905–6	8	289	− (+)−	−	− (+)	−	−	−+	(+)−	
Shields	1895				−	−	−			−	
Sorbonne					−	−					
Stevens	1905		116				−80% + 6%			−57% +29%	
Weber	1910		Few	−	+	−		−		−	+
Weld	1912						−90%				
Wundt								−			
Brain volume											
Berger	1904–7	Few	Few	+−	−	±	+	0		+	
Mosso	1879	3		+	+	+	+				
Shepard	1905–6	2	180	(+)(±)+	+	+	+		+−	+	
Weber	1910	Few	Few			−				+	+
Intestinal volume											
Weber	1910	Few	Few	+	−	+				+	−

+ Increase.
− Decrease.
+− First an increase, then a decrease.
(+)− A temporary increase followed by a decided decrease.
± Now an increase, now a decrease.
(+) / − In some cases an increase, usually a decrease.
0 No change.

arithmetical problems in connection with other experiments. Fourteen experimenters reported vasoconstriction preceded, in a few cases, by a brief vasodilatation. Five, all careful experimenters, found sometimes vasodilatation, sometimes vasoconstriction. Anderson was the only experimenter who found more vasodilatations than vasoconstrictions. His results seem the most trustworthy, however, because he used periods of work long enough to secure true reactions; he performed the same experiment upon thirty subjects and could thereby eliminate individual differences; he experimented upon more kinds of mental work than all the other experimenters combined and he performed several times as many experiments as all the other experimenters combined. We may conclude, therefore, that mental work sometimes causes vasoconstriction in the peripheral arteries, but more often vasodilatation. Weber found that mental work occasioned vasodilatation in the intestinal arteries, and three experimenters found that it caused vasodilatation in the cerebral vessels, as might be expected, since blood always flows to an organ which is being actively used.

Angell and Thompson (3) found that a steady strain of attention caused little if any vasomotor change, and that every break or shift of attention caused vasoconstriction, the amount of constriction being roughly proportional to the intensity of the emotion or stimulus that broke in on the attention, and the amount of surprise involved. My results confirmed theirs, as do the results I have seen everywhere in the literature.

Summary of results obtained by other experimenters. The results of the many experimenters reported in table 1 show that those sensory stimuli (whether agreeable or disagreeable), especially shock, which cause a break or shift of attention, occasion vasoconstriction in the periphery and vasodilatation in the brain, the vasomotor shift being the most noticeable after intense, unexpected stimuli, and the least marked after weak stimuli.

Mental and physical work, which call forth a steady strain of attention, often cause a temporary vasoconstriction in the peripheral arteries due to the shift of attention to the work; then, if the work continues, it brings about a very gradual vasomotor shift, much less noticeable than that occasioned by sensory stimuli, more often vasodilatation than vasoconstriction in the peripheral arteries, and always vasodilatation in the cerebral vessels.

Summary of results obtained by John F. Shepard. In *Organic Changes and Feeling* (29), Shepard reported the results of his experiments on

the peripheral volume changes of six normal subjects. Three forms of plethysmograph were employed at one time or another, Zimmerman's modification of Lehmann's plethysmograph, the Hallion-Compte air plethysmograph and the finger plethysmograph described by Lombard and Pillsbury were employed whenever it was possible, and a piston recorder was used with each plethysmograph. The thoracic breathing was registered by means of a Sumner pneumograph connected with a Marey tambour. Among the stimuli used were agreeable and disagreeable smells and tastes, colored lights, deep and shrill whistles, chords and discords on tuning forks, music on the violin and zither, noises, attention to counting marks or a minimal sound, to a touch or to a multiplication, recalling of emotional experiences, listening to amusing reading, etc. The subject's arm rested in the swing which held the plethysmograph. A normal record was run for some time before any stimulus was given and time was allowed for recovery after each stimulus. In some of the experiments the subject and experimenter were in different rooms.

Shepard found that the reaction was almost invariably vasoconstriction, preceded in a very few cases by a temporary vasodilatation. Out of 200 reactions, 187 gave a fall, 7 gave a rise which was probably due to changes in breathing and not directly to the stimulus, and 6 gave a rise followed by a fall, in 3 of which the fall was much more marked than the rise. Twenty-two cases of strain gave a fall in volume; relaxation gave a rise. Agreeable and disagreeable stimuli gave the same reactions.

About 150 clear records were obtained simultaneously of the changes in volume of both the brain and the periphery of a laborer of fully average intelligence who had met with an accident which necessitated the removal of a piece of the skull bone, roughly 8 by 6 cm. in area, on the right side of the head near the Rolandic region. A brain plethysmograph was made by attaching a piece of cork, cut to fit the dip over the trephine, to the rubber membrane of the tambour; this was held on the head by means of two bandages. The same stimuli named above were employed and it was found, in general, that all agreeable or disagreeable stimuli, all sensory attention or attention to arithmetical problems, all agreeably exciting light or music, all depression, and the strain of expectation when a neutrally toned stimulus was announced before it was given, gave a fall of volume of the hand with smaller pulse and more rounded dicrotic, and rise of volume of the brain with larger pulse, often with dicrotic raised in position and made sharper.

With relaxation, there was at first quite often a fall of the hand volume and rise of that of the brain, then a gradual increase of the hand and a decrease of the brain to normal. With strong stimuli, the reaction often had a double character; the volume of the hand increased first with smaller pulse and then fell quickly to a much lower level with rounded pulse while that of the brain increased at first with large pulse, then decreased nearly or quite to normal and sometimes showed almost an anacrotic pulse; then finally rose markedly with high pulse and gradually returned to normal. Similar results were obtained in similar experiments both from this subject and another trephined subject, and were reported in *The Circulation and Sleep* (30).

Experimental literature on stammering. I am acquainted with but one plethysmographic study of stammering. That is an article by Fletcher (49). He had as subjects nine stammerers between 14 and 24 years of age, the average age being 17; there were five boys, three young men and one young lady. No attempt was made to make a careful diagnosis of each case, hence the reader does not know whether these cases were primarily mental or physical stammering. The apparatus consisted of a kymograph, a Lehmann arm plethysmograph connected with a tambour recorder, two Sumner pneumographs for the registration of both chest and abdominal breathing, connected to tambour recorders, a Jacquet chronometer, a Rousselot microphone, a Deprey d'Arsonval galvanometer, an adjustable reading rack and cards on which were printed various selections of prose and poetry. No attempt to minimize arm movements by a swing or otherwise was mentioned, yet stammerers constantly move their arms while stuttering.

The breathing results are trustworthy and were as follows: The characteristic normal rest-breathing curve showed inspirations and expirations of approximately the same length, and the thoracic and the abdominal curves were approximately synchronous in phase. In general, the stutterers presented no permanent peculiarities of breathing unrelated to the function of speech. There were apparently as many varieties of breathing peculiarities among stutterers as there were varieties of stuttering. Breathing abnormalities tended to become stereotyped in certain forms for certain individual stutterers; they appeared often as temporarily adopted expedients to help out in the beginning of speech. The thoracic curve seemed to be more sensitive to mental disturbances than the abdominal curve. In many cases the thoracic and abdominal curves tended to approach each other, often to the point of touching; this sometimes continued through-

out the speaking interval. In many cases breathing disturbances appeared before the speaking interval and continued for as long as eight seconds after it. The ratio of the time of inspiration to that of expiration was found to be 0.217 second during normal speech and 0.535 second during stuttering. The expiration interval averaged 24.6 seconds during stuttering, of which the vocalization interval occupied but 9 seconds; the record of normal speech showed that of an average expiration interval of 26.8 seconds, 25.6 seconds were utilized in vocalization. There appeared to be an efficiency of 90 per cent with normal speech as compared with an efficiency of 36.5 per cent with stuttering.

Shepard's plethysmographic results were as follows: There was a marked attention drop (constriction) in 73 per cent of the cases where the stutterer was told to speak or read, this drop being greater in the latter case. Immediately after the attention drop there began, in 90 per cent of the records, a rise (vasodilatation) which usually lasted until the subject ceased stuttering; this amounted to a rise of 5 cm. in 31 seconds in one extreme case. In 62 per cent of the records, this rise was interrupted by irregularities in addition to those due to breathing and movement. When subjects were asked to imagine themselves in situations in which they would be likely to stutter, the curve showed slight vasomotor constrictions in certain cases, never vasodilatation. Both the amount of the general rise and the distortions of the plethysmograph are correlated with the degree of severity of the stuttering.

The speech of the stutterers was attended by an abnormal acceleration of the pulse rate. At a point in the period just prior to the speaking interval, the pulse rate averaged 90.2, ranging from 72 to 120; at a point at the beginning of the speaking interval, the pulse rate averaged 99.8, ranging from 78 to 126; and at a point at the close of the speaking interval, the pulse rate averaged 98.6, ranging from 72 to 129. It will be remembered that the normal pulse rate in adults ranges from 70 to 75.

Apparatus. The apparatus used in these experiments was practically the same as that used by Dr. John E. Anderson, and consisted of a Zimmerman kymograph, a Verdin pneumograph, a Sumner pneumograph, a Marey tambour, a Lehmann plethysmograph, a finger plethysmograph, a piston recorder and two small electromagnets.

The kymograph was the regular Zimmerman model with an extension which permitted the use of smoked paper 2.5 m. long by 16 cm. wide. This kymograph was practically noiseless at the low speed at which it was run. As few of my records required smoked paper over

8 cm. wide, I usually went twice around each record; this enabled me to perform four consecutive experiments of approximately an hour each without changing records.

Zimmerman's modification of Lehmann's arm plethysmograph was used in the earlier experiments. A long-sleeved rubber glove was substituted for the blind sleeve, and a movable but tightly fitting round block of wood was placed vertically within the plethysmograph near the end furthest away from the part where the rubber glove was attached. The plethysmograph was attached firmly to a swing suspended from the ceiling. An elbow-rest, which could be moved along a groove in the swing and clamped at any point, kept the subject from withdrawing his arm; a leather strap, passed over the subject's forearm near the elbow and beneath the swing, kept the subject from raising his arm out of the elbow rest and thus withdrawing it slightly from the plethysmograph; and the vertical stick within the plethysmograph, when lightly clasped, kept the subject from thrusting his arm further into the plethysmograph. Thus the only motion which could seriously affect the record was a motion of the arm within the plethysmograph, a movement which could usually be detected on the record because of its characteristic abrupt rise or fall. The curve often returned quickly to normal after such movements and was then little affected; if it did not return to the normal level, that part of the record was always discarded. The subject was instructed always to clasp the stick lightly, because it was found that there was a tendency to relax the grip now and then when the stick was clasped tightly, in which case the recorder seldom returned to the normal level. He was told, also, to relax his muscles and to keep his hand in as comfortable a position as possible.

After the subject put on the rubber glove of the plethysmograph but before he thrust his entire forearm into the plethysmograph, the operator filled the plethysmograph with water heated a few degrees above bodily temperature and screwed on the glass tube to the top of the plethysmograph. This glass tube was 24 cm. long and, when nearly filled with water, served to press the water tightly against the subject's hand at every point. The subject then thrust his arm into the chamber until he clasped the stick lightly, and the operator adjusted the elbow-rest and strap and added or took water from the glass tube until it was filled to within 2 cm. of the top. The top of this glass tube was connected by thick-walled rubber tubing, through a syringe and outlet to the air, with the piston recorder, as described on page 296 in connection

with the finger plethysmograph. The piston of the recorder used with the arm plethysmograph was cut with shears from a thin sheet of brass and was therefore not perfectly round. A strip of bond paper with a fine wire inserted at the outer end was used as a recorder. The lever of the recorder was the same as that to be described later, but it worked up and down between the two springs made of 36 gauge wire (0.019 cm. in diameter) used by Doctor Anderson, touching the springs about 2 cm. from their axes. The lever worked at every point against the delicate springs so that when the pressure against the piston was relaxed, the piston automatically returned to its normal level, thus counteracting the effects of inertia and momentum. By turning the strip of brass to which the springs were attached about the axis, the springs could be set at any distance from the axis of the recording lever; hence the size of the recorded pulse could be regulated for differences in each subject. As this arm plethysmograph could not be used with stammerers, since they moved their arms continuously while stammering, the finger plethysmograph was substituted in my later experiments, in order that I might compare more readily the vasomotor changes in stammerers with those in normal speakers.

The finger air plethysmograph was made in the shop at the laboratory and was a modification of the one used by Lombard and Pillsbury and described in volume III, pages 191–193, of this Journal. The air chamber consisted of a brass pipe 10 cm. long and 2.5 cm. in diameter with a metal disk, pierced by a pipe with a bore 4 mm. in diameter soldered to one end, and a rubber diaphragm 0.026 inch thick, with a small hole in the center, fastened to the other end. This air chamber was placed inside of, and projected 1 cm. in front of, a second watertight pipe, 12.5 cm. long and 4.5 cm. in outside diameter, with two outlets through which water of constant temperature might pass and keep the air in the air chamber at constant temperature. As there was no running water in the room, the water chamber was not used. This plethysmograph was firmly attached to a swing suspended from a rod 43 cm. above it. When the subject thrust the middle finger of his right hand through the hole in the rubber diaphragm until it could go no farther, the operator adjusted the same elbow rest and strap used with the arm plethysmograph. Then no ordinary movements could affect the record other than movements of the finger within the plethysmograph; these could usually be readily detected because of their characteristic abruptness. As the recorder usually returned to its normal level after such movements, they seldom affected the record;

the few parts of records in which the lever did not return to its normal level were discarded. A thick-walled rubber tube connected the finger plethysmograph with a metal T-tube. One end of the T-tube led to a glass syringe 10 cm. long with a piston 13 mm. in diameter; the other end of the T-tube led through a thick-walled rubber tube of the same diameter to a second similar T-tube. One end of this second T-tube led to the open air and was always open when no experiment was in progress, but always closed by a metal clamp when the experiment was in progress; and the other end led to the piston recorder. As the syringe was airtight, it was possible, by moving the plunger, to alter the amount of air in the system of tubes connecting the finger chamber with the recorder and thus place the lever in a horizontal position when it was working too high or too low because of changes in temperature or arm movements.

Of the many forms of apparatus which have been devised for recording minute vasomotor changes, a piston recorder, properly made, is the most delicate. As the piston recorder which I used with the arm plethysmograph made no record when attached to the finger plethysmograph, it was necessary to modify this recorder.

I selected a glass T-tube having a bore 5.2 mm. in diameter which was almost round. One of the opposite ends was sealed; the other was ground or lapped out so as to be perfectly round its full length for the piston to work in. A perfectly round piston, which fitted the piston barrel closely and yet worked up and down and turned almost over in the tube without binding, was cut from a strip of celluloid 0.026 inch thick. The celluloid piston was practically as stiff as one made of sheet brass, yet soft enough to be cut by a hollow round steel punch. This was a decided advantage because brass has to be cut with shears and will necessarily have little corners which will permit air to escape and injure the record. The point of a stiff steel pin, 0.028 inch in diameter, was passed through the center of this piston and slightly riveted so that it could not pull off; the head was cut off and a hook was made of this end. When this hook was passed through a hole which was drilled in the brass lever and which just fitted it, this pin made a very satisfactory piston rod 4.5 cm. in length.

The lever of the piston recorder was the one used by Doctor Anderson, excepting that his writing lever made of bond paper tipped with a fine hair was replaced by a very thin strip of celluloid, 0.01 inch in thickness, which was shaped at one end to form a sharp writing point and was attached by varnish to the sheet brass part of the lever. The

celluloid lever was almost as flexible horizontally as the bond paper but it had greater vertical rigidity, could not be bent by accident as could the paper and was not affected by air currents as was the paper. All of the lever but the celluloid tip, which was 54 mm. long and 4 mm. wide, was cut from a piece of sheet brass 0.018 inch thick. It was made from a strip 13 cm. long and 4 mm. wide, and one end was turned twice at right angles and fastened to its axis by the pin *MN* as shown in figure 1. A small piece of lead, *L*, was placed on the arm of the lever on the opposite side of the axis from the writing point; thin shavings were cut from this lead until the piston would stay at any point of the barrel where it was placed, before oil was dropped into the barrel or the writing tip touched the drum of the kymograph. Thus the pressure on the lower part of the piston had to work only against the weight of the drop of heavy machine oil on the top of the piston and the friction of the joints and of the writing tip of the lever on the smoked paper; this proved just sufficient in an upward thrust to diminish the extra upward movement due to momentum from the strong upward thrust at the beginning of each pulse beat. As the weight of the drop of oil and the friction on the smoked paper act in opposite directions when the piston is falling, the lever quickly returned to normal after each pulse beat. I watched the piston closely during all experiments and seldom saw a bubble of air escape unless a little oil hardened on the sides of the piston-barrel and did not permit the piston to move freely. I avoided this by frequently removing with alcohol the oil which is bound to adhere to the sides of the barrel as the piston works up and down. What little oil ran down the sides of the tube collected at the sealed end directly below the piston-barrel and did not interfere with the pulse waves which came in through the third horizontal arm of the T-tube from which the recorder was made. The piston could move an inch above or below the position it assumed when the lever was horizontal, without catching or binding.

The springs used with the arm plethysmograph were discarded as the changes in pressure due to the minute vasomotor changes in a single finger were not sufficient to move them. The writing point was 12.5 cm. from the axis of the lever and the piston rod was attached to the sheet brass part of the lever 3 cm. from the axis; hence the motion of the piston was magnified a little more than four times on the record. When the piston rod was attached nearer to the axis of the lever, the piston was apt to bind in its barrel, and the piston rod often touched the sides of the barrel and bound when the lever moved much above or

below the horizontal position. If the lever were much longer, the friction between the writing tip and the drum would increase and interfere with the free working of the piston within its barrel.

The piston recorder was fastened securely to the end of the round horizontal rod *AB* by the clamp *A*. This horizontal rod was fastened by a clamp (not shown in the figure) to a vertical rod which was firmly attached to the horizontal revolving arm of a stand which could be raised or lowered by turning a screw, and placed nearer or farther away from the drum of the kymograph by moving it gently with the hand. At a convenient distance from the end of the horizontal rod *AB*, a vertical hole, *B*, was drilled to fit the smaller round rod *BQC*

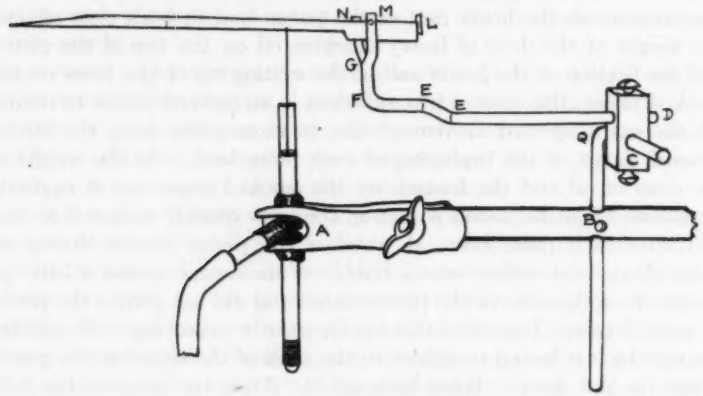

Fig. 1

which was turned at right angles at *Q*. Two horizontal holes, *C* and *D*, were drilled at right angles to each other in the short square rod *CD* to fit the round rods *BQC* and *DEFG*. Rod *DEFG* was turned horizontally at right angles twice at *E* and vertically at right angles at *F* and was split down the middle and bent at *G* to hold the axis pin *MN*. Screws clamped the rods firmly together at *B*, *C* and *D*. The piston could be raised or lowered to any point of its barrel by loosening the screw *B* and raising or lowering the vertical rod *BC*. The piston rod could be brought over the center of the piston barrel and made parallel to this by loosening screws *C* and *D*, moving the rod *DEFG* and revolving it about the axes *QC* and *DE*. When the lever is horizontal, the apparatus should be so adjusted that the piston-rod will lean

slightly toward the writing point because it swings away from the writing point with either an upward or downward movement, and such an inclined position keeps the average position of the piston-rod more nearly vertical and prevents binding.

This piston recorder satisfied the requirements outlined by Lombard and Pillsbury on page 187 of volume III of this Journal. The piston was thin so as to minimize the friction in the piston barrel. It was of small diameter and so connected to the piston rod and the lever that it could not bind and could tilt but little. The movable parts were as light as possible in order to avoid the effects of inertia and momentum. The piston moved freely in the tube and yet followed readily the movement of the lever, which not only described an arc as it moved up and down, but moved a little to one side or the other when the writing point passed over an uneven place on the smoked paper, so as not to keep exactly over the center of the piston. As the compressibility of the air below the piston requires that the lever should write on the smoked surface of the kymograph with the least possible friction, the lever was made flexible so as to yield to the inequalities in the recording surface and at the same time be sufficiently springy to maintain a continuous, gentle pressure on the drum of the kymograph.

The lever of the piston recorder worked up and down in a vertical plane parallel to the drum of the kymograph. The writing point should be tangent to the drum when the piston is about 15 mm. above or below the position it assumes when the lever is horizontal. If the writing point is tangent when the lever is horizontal, it will not, when it rises or falls, touch the drum more than a few millimeters, unless, when horizontal, it presses too tightly against the drum to make an accurate record.

To test the effect of this change in pressure on the drum as the writing lever approaches and recedes from the drum, and to see what change in volume of the finger corresponds with the rise or fall of 1 mm. on the record, I constructed the following piece of apparatus. With thick-walled rubber tubing I connected one end of a glass capillary tube, 10 cm. long, with a bore 2.25 sq. mm. in cross section, with the part of the T-tube which led to the air in my former experiments. I attached about 30 cm. of the same rubber tubing to the other end of the capillary tube and passed it between a board and the table. I clamped the capillary tube in a vertical position close to a scale and filled it with mercury. By tightening or loosening the clamp which pressed the board against the table, I could raise or lower the height of

the mercury in the capillary tube. As I made the volume of air from the mercury to the T-tube the same as that in the tube which formerly led to the air, and placed in the plethysmograph a wooden finger of the same volume as my own finger, the volume of air in the plethysmograph and connecting tubes was the same as it was in my former experiments; the wooden finger, of course, could not move. When this apparatus was connected, I lowered the mercury by eight equal amounts and then raised it by the same number of equal amounts, running the kymograph for an instant between each rise or fall. I did this several times. Then I measured the height of each little rise or fall on the smoked paper and found that these all averaged the same length for a given position of the lever, and that the position of the writing lever therefore made no difference in accurately recording the vasomotor waves when it just touched the drum when the piston was raised just a little more than 15 mm. above the horizontal position of the lever. When the writing point just touched the drum when the piston was raised considerably more than 15 mm. above the horizontal position of the lever, the pressure of the writing point on the drum became so great, when the lever was horizontal, that the mark then traced was much shorter than that traced when the writing point just touched, although both represented the same change in volume. It is necessary, therefore, to move the writing point nearer the drum if the writing point rises or falls much more than 15 mm. from the horizontal position, and to move it away the same amount when it returns.

I repeated this procedure when the plunger of the syringe was at one end of the syringe and again when it was at the other end, and found that no difference in amplitude could be detected in the record for like changes in the column of mercury at these two positions; hence the differences in the position of the plunger of the syringe in different experiments, and differences in the sizes of the fingers of different subjects which were in all cases less than the volume of the syringe, do not affect a trustworthy record of the vasomotor changes.

I found that a rise of 1 mm. on the records always denoted an increase in volume of the finger of 3 cu. mm., and that a fall of 1 mm. on the record always registered a decrease in finger volume of 3 cu. mm., for changes not exceeding 15 mm. above or below the horizontal position of the lever, and for all changes if the lever is moved a little toward the drum when the rise or fall exceeds 15 mm. and is withdrawn the same amount when it returns to that point.

As my piston recorder appeared to move instantly when I moved the plunger of the syringe, and as the latent period of a similar piston recorder tested by Lombard and Pillsbury was found to be but one one-hundredth of a second, I did not repeat their test. A latent period of less than a second would not affect my results.

As Lombard and Pillsbury found by careful experiment that the curve traced on the record by a piston recorder similar to mine was a true record of the normal pulse curves from the finger, and does not originate in the recording mechanism, I shall assume the same to hold true with my recorder without repeating their elaborate test experiment.

The thoracic breathing was obtained either by a Sumner pneumograph or by a Verdin pneumograph. The connecting chain was passed just below the subject's armpits. As the Verdin pneumograph frequently turned over during the stammerers' contortions and gave a less magnified record than the Sumner pneumograph, the Sumner pneumograph was used in practically all of the records so that the records of normal speakers and stammerers might be more easily compared.

The breathing record was obtained by a writing lever attached to a Marey tambour 2.5 cm. in diameter. The lever was well balanced and consisted of a bond paper writing arm with a very fine wire at its outer end. As the tip of the writing wire was 14.3 cm. from the axis of the lever, and the lever was attached to the rubber diaphragm of the tambour 1.2 cm. from the axis of the lever, the movements of the tambour were magnified twelve times. As the rubber diaphragm of the tambour was uppermost in my earlier experiments, the top of the curve in these records represents empty lungs. As the rubber diaphragm was inverted in my later experiments, including most of the work with the finger plethysmograph, the top of the curve represents full lungs in these records; this seemed more logical and made the records of the stammerers easier to read. Unless specifically mentioned to the contrary, the top of the records here reproduced will represent full lungs and the bottom of the records empty lungs.

The breathing record is a valuable check on the plethysmographic curve as it shows what sudden changes in the plethysmographic record are caused by deep breathing, yawning, laughter or sneezing. The breathing record was especially useful in comparing the breathing of stammerers and normal speakers while reading the same passage. I used the pneumograph only for this purpose.

A thin celluloid writing lever, pointed at the outer end, was attached to each of the electromagnets. The upper magnet was connected with a two-way switch. One side of this switch was connected with a time key which could be pressed by the operator and with another key which could be pressed by the subject. The other side of the switch was connected with one side only of a large electric pendulum making one stroke a second; this caused the magnet to click and mark the record every two seconds. When the clock happened to be out of order, I used a stop watch and made marks on the record at known intervals by pressing the operator's time key; as the kymograph rotated at practically a uniform rate, the time could be interpolated pretty accurately between any two points on the record. The lower electromagnet was connected *a*, with the operator's signal key; *b*, to a key which could be pressed by the subject; *c*, in series with a signal light in front of the subject through a switch near the operator and *d*, in parallel with a faint signal buzzer near the subject through another switch near the operator; thus signals were recorded on the record the instant they were given, unless the operator had to use one hand to make the stimulus, as in blowing a whistle, and press his signal key with the other hand, when there was apt to be an error of a fraction of a second. Such an error, however, was too small to influence the results, as I did not try to make any measurements in fractions of a second, whole seconds being accurate enough for the purposes of these experiments.

Procedure. The arrangement of the apparatus used is shown in the accompanying photograph. The writing points of the four recorders were adjusted to write in the same vertical line when the recording levers were horizontal. The time recorder was at the top, the operator's signal recorder next below, the tambour recorder which traced the breathing curve below that and the piston recorder, which traced the vasomotor changes, was at the bottom. I adjusted the swing at a comfortable height for the subject's arm and then adjusted the elbow-rest. Next, when the subject's lungs were half full of air, I put on the pneumograph so that its writing lever would be above the horizontal position as much of the time as it was below it, and thus work more nearly straight up and down. After requesting the subject to remain quiet until the experiment was over, I then closed the outlet from the tube which connected the finger plethysmograph with the air. When the writing point of the piston recorder remained still for a few seconds, showing that the temperature within the tubing had

ceased to rise noticeably, I started the kymograph. As I was interested in the volume changes rather than in the shape of the pulse, I ran the kymograph at low speed; hence little, if any, pulse waves are noticed in these records. Very distinct pulse waves appeared in some subjects, however, whenever the kymograph revolved at high speed. I ran the kymograph for about a minute before any stimulus was given, in order to obtain a normal record from which to measure the reactions to the stimuli that followed; during this normal period I asked the subject to remain as calm and as quiet as possible so that no emo-

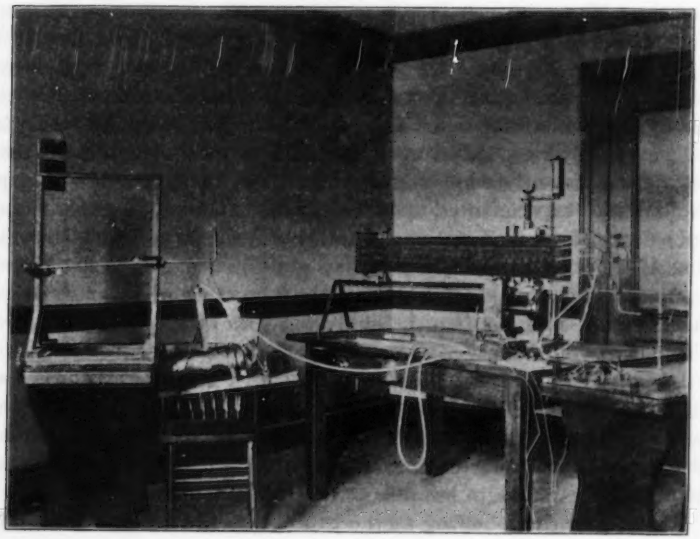

Fig. 2

tions or movements would affect this normal reference line. After the stimulus was given, at least a minute was allowed for the piston recorder to return to its normal level, unless it returned much sooner. Sometimes several stimuli were given in quick succession; sometimes stimuli were given at intervals of a few minutes; sometimes but one or two stimuli were given in an entire period of an hour. The kymograph was stopped at intervals to secure reference lines from the four recorders, to make sure they were keeping in a vertical line. The subjects were allowed to rest and to move whenever they became tired

or uncomfortable or whenever their records showed frequent arm movements; but first they always asked the experimenter's permission to move. A screen was always placed between the experimenter and the subject so that the subject could not see what stimulus was next to be given and the operator was careful not to make a noise when handling stimuli.

I had, as subjects, thirteen graduate students or college seniors and ten stammerers. The normal speakers were experimented upon once each week at the same hour, between 9 a.m. and 1 p.m.; three came for two months, six came for four months and four came for eight months. Nine were men, four were women. The stammerers, all young men with the exception of one boy, came at irregular intervals, frequently in the evening after a hard day's work; some came but once or twice. I selected from about one hundred students at the Boston Stammerers' Institute those who could be depended upon to stammer whenever they were called upon to read and those who did not move their arms violently while stammering; a large majority of the pupils could not be experimented upon. As most of the pupils experimented upon learned to read without hesitancy within two weeks from the time they entered the Institute, they could not long be experimented upon as stammerers. Some came to the laboratory at various intervals during their cure, so that I could study the vasomotor changes at different parts of their cure and compare them.

I gave, in all, about 1000 stimuli. A large majority of the records had to be discarded because they were obscured by arm movements. Less than 1 per cent of the reactions to stimuli with the Lehmann plethysmograph were comparatively free from arm movements. About 150 reactions to stimuli were retained and measured.

I employed the following method to obtain the fall or rise in millimeters of the recording needle during an emotion, the time in seconds from the stimulus to the lowest or highest point of the curve traced immediately after the stimulus was given and the time in seconds from this lowest point to the return of the needle to normal. I placed a transparent celluloid triangle over the record and moved it around until I got one edge over what seemed to be the route the needle would have taken if the stimulus had not been given, allowing for all arm movements as far as possible. If I did not wish this part of the record for reproduction, I then drew a line with a fine needle along this path which the recording needle would have traced if no stimulus had been presented; if I wished to preserve the record for publication, I left the

triangle lying there. The line traced by the needle was never parallel to the time line, for the tube was usually either warming up a little or cooling down a little even when I tried to keep the room temperature constant. The lowest or highest point of the curve was obtained by placing a second triangle touching the first before it was moved from this position, and sliding the first triangle along the second until it became tangent to the lowest or highest point of the curve. This position of tangency was marked on the record with a sharp needle. I next placed a triangle along reference lines taken at intervals on records to ascertain whether the ends of the piston recorder, tambour recorder and two electromagnet recorders were keeping in a straight vertical line, so as to be tangent to the reference marks traced by the operator's key and the piston recorder. I placed a second triangle touching the first and slid the first along the second, marking on the time line the point corresponding with the instant the stimulus was given, the point corresponding to the moment when the fall of the needle was at its maximum and the point corresponding with the instant when the needle returned to normal. By counting the time notches between these marks, I obtained the two times referred to above. By placing one leg of a right triangle on the time line with the other leg passing through the lowest point of the plethysmogram, I was able to measure along the latter leg, with a millimeter scale, the drop to the lowest point of the curve from the standard line the needle would have traced if no stimulus had been given. If an arm movement was noticed and the needle did not return to this standard line within a minute from the time the last stimulus was given, that part of the record was discarded. Arm movements could usually be readily distinguished because of their abruptness. If a record contained arm movements at intervals of an inch or two, I discarded that part of the record, because these movements showed that the subject was then too nervous to keep still, so that it was impossible to get an accurate reference line.

Among the stimuli employed at one time or another to arouse emotions in both normal speakers and stammerers were the following:

1. A shrill whistle.
2. A loud crash, sometimes in front of the subject, sometimes behind him.
3. A sudden yell from the operator.
4. The report from a cap, sometimes placed in the book the subject was asked to read so that it would go off when he opened the book.

5. The sudden entrance of a third person into the room.

6. A loud sneeze by the operator.

7. The subject read silently an exciting story or gruesome description and pressed a key whenever he experienced an emotion.

8. The operator let down an artificial spider on a string close to the subject's head.

9. The subject was directed to take an unpleasant unknown object, such as a rubber snake, out of a cloth bag.

10. The operator threw a light, artificial rubber snake at the subject.

11. The operator aimed at the subject a noisy, self-propelled red balloon.

12. The operator placed close to the subject material for a bonfire, oiled it in his presence and told him to open the match box given him at a signal and light the fire. (When the subject pressed the catch to open the match box, a spring made it fall apart.)

13. A large mechanical bug crawled toward the subject.

14. The operator set near the subject a mechanical frog adjusted to jump six feet into the air about half a minute after being placed.

RESULTS

As arm movements affected most of the curves traced by the piston recorder when it was attached to the arm plethysmograph, no records made with the arm plethysmograph are included in the following tables. Although these curves could not be accurately measured and compared, they confirmed the results tabulated below.

All of the curves showed vasoconstriction in the finger both during stammering and during the emotional disturbance induced by a loud, sudden stimulus such as a shrill whistle, which will hereafter be called *shock*. A small proportion of the curves showed a slight rise for three or four seconds, followed by a decided fall of considerable duration.

In all of the tables, "fall in millimeters" was measured from the path the writing needle of the piston recorder would have taken had no stimulus been given, to the lowest point of the curve immediately following the stimulus. If, during a long period of stammering, there was a temporary rise followed by a greater fall than the first one, the greater drop was the one measured. Figures 4 and 6 show the method of measuring these falls. "t" was measured in seconds from the instant the stimulus was given to the time when the recording needle

first attained the maximum drop. "T" was measured in seconds from the end of "t" to the time when the recording needle first returned to the path it would have taken had no stimulus been given, unless the time of maximum drop occurred during a long period of stammering, when "T" was measured from the end of the stammering period instead of from the end of "t." Under each of the headings "fall," "t" and "T," the maximum, the average, and the minimum are given, so that individual differences may be studied.

Table 2 shows the vasomotor changes in seven normal speakers during shock induced by the six kinds of stimuli named in table 3. These subjects are listed in the order of decreasing magnitude of fall. This

TABLE 2

Vasomotor changes in individual normal speakers accompanying shock

SUBJECT	NUMBER OF STIMULI	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
		Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
E	5	26	21	15	16	10	5	100	56	33
G	15	20	13	5	22	11	2	95	35	7
H	12	19	10	5	43	14	3	98	36	13
A	9	13	8	5	44	23	6	75	47	16
J	13	15	8	3	28	14	8	98	39	10
F	9	20	6	2	70	31	7	64	31	11
D	4	9	4	1	51	32	23	64	37	12
Total	67									
Average		17	10	5	39	18	8	85	39	15

table shows that these subjects are also arranged approximately in the order of increasing length of "t," but in the order of decreasing length of "T" the correlation between the average "Falls" and "t's" being −0.775, and the correlation between the average "Falls" and "T's" being +0.908. The order is the same, in practically every case, both for the maximum and for the average. This table shows, therefore, that for the subject who has a relatively large fall, the time of fall is relatively short; for example, subject E's average fall of 21 mm. occurred in an average of 10 seconds, whereas subject D's average fall of 4 mm. occurred in an average of 32 seconds. This table shows, furthermore, that those subjects who experience the largest falls also have the slowest recovery and the individual cases show that the greater the fall,

the slower is the recovery. A study of the individual cases shows that subjects do not vary any more from day to day than they vary during a single hour. As table 3 indicates, these differences for the same

TABLE 3

Vasomotor changes in normal speakers accompanying shock brought on by stimuli of different intensity

STIMULUS	NUMBER OF STIMULI	NUMBER OF SUBJECTS	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
			Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
Gun	5	4	26	18	7	34	14	2	100	63	10
Whistle	10	5	18	9	5	24	12	6	80	30	15
Threw snake	5	3	15	10	6	38	14	6	60	40	13
Crash	19	7	23	9	1	51	19	2	98	34	11
Yell	3	3	7	6	5	70	31	10	40	29	23
Spider	7	5	11	7	2	23	15	6	72	31	12
Total	49	7									
Average			17	10	4	40	17	5	75	36	14

TABLE 4

Vasomotor changes in stammerers accompanying shock brought on by stimuli of different intensity

STIMULUS	NUMBER OF STIMULI	NUMBER OF SUBJECTS	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
			Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
Gun	7	5	50	25	9	16	10	5	110	80	38
Whistle	11	5	30	19	8	20	12	7	88	44	19
Threw snake	5	5	30	15	0	19	13	9	65	48	37
Crash	6	5	23	14	9	65	21	6	93	50	25
Yell	6	4	25	16	10	19	12	8	58	43	30
Spider	4	4	13	8	3	11	7	4	34	28	21
Total	39	10									
Average			28	17	6	25	13	6	74	50	28

subject are due to the intensity of the stimuli and to the degree of surprise involved. All these tables show decided individual differences.

Table 3 shows the reactions of the seven normal speakers listed in table 2 to six stimuli of different intensity. Table 4 shows the reac-

tions of ten stammerers to the same stimuli. These tables show that the more intense the stimulus is, the greater is the vasoconstriction and the slower is the recovery. A close examination of the individual curves showed that the more unexpected the stimulus is, the greater is the vasoconstriction and the slower is the recovery; this is seen when the reaction to the first gun fired is compared with that to later reports of the same gun, or when the whistle is blown several times on the same morning and the successive reactions are compared, or when the introspections of the subjects tell which records to compare. These tables also show that stammerers as a class experience greater and more rapid vasoconstriction and slower recovery in shock than do normal speakers.

TABLE 5

Vasomotor changes in individual stammerers accompanying shock

SUBJECT	NUMBER OF STIMULI	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
		Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
Web	7	50	26	8	11	9	5	100	59	29
Wi	7	30	23	9	12	10	7	110	66	35
Ad	4	23	20	14	16	15	11	93	65	30
De	3	23	14	8	20	19	19	58	41	19
Li	5	18	12	10	9	8	6	65	42	30
An	4	15	12	10	14	10	7	75	38	22
Fa	1	12	12	12	65	65	65	25	25	25
Bo	2	8	6	3	13	12	11	42	34	27
Total	33									
Average		22	18	9	20	13	16	71	52	27

Table 5 shows the reactions of eight stammerers to shock produced by the six stimuli listed in table 4. This table, like table 2, shows that those stammerers who experience a relatively great vasoconstriction also require a relatively long time for recovery, the correlation between the average "Falls" and "T's" being +0.698. It shows, also, that those subjects who experienced relatively great vasoconstriction tend, like normal speakers, to have relatively rapid vasoconstriction, although the correlation between the average "Falls" and "t's" is but −0.039. This is not so striking as for the normal speakers, because fewer stimuli were given and the stammerers had not been trained to keep their arms still, as had the normal speakers; nevertheless this relationship is noticeable in the first four subjects listed. The stam-

merers experienced 80 per cent greater vasoconstriction than did the normal speakers, and this attained its maximum in two-thirds the time it did in normal speakers. The time of recovery was 23 per cent longer for the stammerers than for the normal speakers. The maximum fall for stammerers was almost double the maximum fall for normal speakers, and the minimum time of recovery was nearly three times as long for the stammerers as it was for the normal speakers. Shock, therefore, is a greater emotional disturbance in the average stammerer than it is in the average normal speaker. An examination of the individual reactions shows clearly that the greater the vasoconstriction is, the slower is the recovery in practically every case. The relation between the magnitude of the fall and the rapidity of the fall is not so definite when we study the individual curves of normal speakers and stammerers. In about half of the subjects, the time of fall is about the same for both large and small falls; in others it is roughly proportional to the fall, in others it is roughly inversely proportional to the fall, and in still others there is no relationship whatsoever.

Table 6 shows the vasomotor changes of the eight stammerers listed in table 5 during stammering. For these eight stammerers as a class, the average drop was 20 per cent greater in shock than in stammering and the average time of recovery was 2 per cent longer than in stammering. For stammerers as a class, therefore, stammering is almost as great an emotional disturbance as shock. If the curves of shock caused by the shots are disregarded from the results it will be seen that stammering produces a greater emotional disturbance than shock, for the average fall is exactly the same and the time of recovery is 16 per cent longer in stammering than in shock. It will be noticed that the subjects are arranged in both table 5 and table 6 in the order of decreasing magnitude of the fall, and that the subjects do not occupy the same places in the two tables. Thus Bo, who was last in table 5, is practically first in table 6. Web, who occupied first place in table 5, has the last place in table 6. With the exception of Fa, who reacted but once to a single stimulus and who can therefore hardly be assigned to a relative postion in table 5, the other subjects occupied about the same places in the two tables. It is clear that the subjects are not in reverse order in the two tables. It simply happened that Web was the slightest stammerer of the eight and was greatly affected by shock, and that Bo, a senior medical student, was one of the severest stammerers of the eight, and was little affected by shock. The correlation between table 5 and table 6 is high, being +0.919 for the "Falls" and +0.897 for the "T's," if Fa is excluded.

A study of the individual records shows that the time of recovery bears no relation to the length of the passage read but is dependent, rather, on how severely the subject stammered while reading the passage, especially at or near the end of the passage. If, in the middle of the passage, the stammerer got a good start, he stammered little and the needle began to rise; if he then met a hard word and either became afraid of it or began to stammer severely, the needle fell; if such a hard word occurred near the end of the passage read, the needle had further to rise, and "T" was lengthened. The time of recovery never occurred during a stammering interval, and it occurred but twice at the end of a stammering interval—once, after a subject who never

TABLE 6

Vasomotor changes accompanying stammering

SUBJECT	NUMBER OF STIMULI	LENGTH OF STAMMERING	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
			Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
Wi	9	60	36	21	12	165	72	24	133	73	42
Bo	7		30	20	11	110	80	20	129	85	40
Fa	6		19	16	14	48	40	25	55	39	20
De	7	121	21	15	9	120	65	40	82	60	34
Li	2		17	12	6	42	37	32	75	48	21
Ad	8	36	17	12	7	54	24	8	80	40	21
An	8	89	18	12	8	94	63	27	48	29	0
Web	6	54	21	11	4	60	25	7	30	23	16
Total	53										
Average		71	22	15	9	87	53	23	79	51	24

stammered while reading after his eighth lesson had been reading aloud, and once when the maximum drop occurred more than a minute before the end of a four-minute reading period in a curve where it was difficult to obtain an accurate reference line. In only two other cases did this recovery take place within twenty seconds; twice Web, the slightest stammerer in the group, recovered in 16 seconds. The recovery was accomplished in from 20 to 25 seconds in nine cases, seven of these being the curves of the two slightest stammerers; in from 25 to 30 seconds in six cases; in from 30 to 40 seconds in six cases; in from 40 to 50 seconds in eight cases; in from 50 to 60 seconds in seven cases; in from 60 to 80 seconds in six cases; in from 80 to 100 seconds in six cases; and in more than 100 seconds in four cases. Thus the recovery

occurred within twenty seconds in but 7 per cent of the stammering curves, and then only in the plethysmograms of the two slightest stammerers and in one other which was in doubt. It took place in from 20 to 30 seconds in 27 per cent of the curves, 73 per cent of these curves being those of the three slightest stammerers. It was completed in from 30 seconds to 1 minute in 37 per cent of the cases, and in more than 1 minute in 29 per cent of the cases. Eighty-one per cent of the latter were the curves of subjects Bo, De and Wi, who were among the four severest stammerers. The worse a person stammers, therefore, the slower is the recovery.

Before compiling these tables, I arranged the eight stammerers in the order of decreasing severity of impediment as follows: Fa, Wi and De, Bo and Li (in doubt, as neither had completed his course at the Boston Stammerers' Institute), Ad, An and Web. It will be seen that the order in table 6 is very much like the order in my estimate as to the severity of the several cases, the correlation being +0.79; hence the amount of vasoconstriction and the time of recovery are important factors in determining the severity of an impediment of speech. As some subjects are more sensitive than others, the ratio of time of recovery from stammering to the time of recovery from shock is a more accurate estimate of the severity of a case, a ratio more than 1.35 being a severe case, a ratio of unity being about an average case and a ratio less than 0.65 being a moderate case. According to this ratio of recovery times, the following order of decreasing severity of impediment is obtained, Bo...2.50, Fa...1.56, De...1.46, Li...1.14, Wi...1.11, Ad...0.62, An...0.61 and Web...0.39, the correlation with my estimated order being +0.76. If another, similar ratio is found, the ratio of the average fall accompanying stammering to that accompanying shock, the following is the order of decreasing severity of impediment: Bo...3.33, Fa...1.33, De...1.07, Li...1.00, An...1.00, Wi...0.91, Ad...0.60 and Web...0.42, the correlation with my estimated order being +0.61, and the correlation with the time of recovery ratio being 0.90. The order is the same in the time ratio and the fall ratio with the exception of An, who comes two places earlier in the latter than in the former. As the magnitude of the fall is affected by the varying pressure of the writing needle on the drum and by finger movements, the time of recovery ratio is the more accurate of the two.

A study of the individual plethysmograms shows that if a stammerer who has been taught to read without hesitancy attempts to read when the peripheral vasoconstriction from shock is at its maximum, he will

stammer severely; if he reads the same passage a minute later after this constriction has ceased, he does not stammer. If a normal speaker is about to speak a word when a shrill whistle is blown, the sound of that word is suddenly lost and he is unable to say it until peripheral vasoconstriction has ceased; that is, he becomes a temporary stammerer. The typical stammering curve remains low throughout the interval of stammering, with occasional temporary increases and decreases in the low volume. When stammerers are partially cured, the amount of peripheral vasoconstriction decreases and the time of recovery decreases. When the stammerer is cured, his record shows no drop unless it has the same brief attention drop that regularly accompanies other shifts of his attention. If stammering begins with an attention drop as normal speech does in some subjects, the stammering curve will remain low throughout the stammering interval while the normal speech curve will begin at once to return to normal and will sometimes rise above normal as shown in figure 9. Subjects who give an attention drop when they commence to read or speak are apt to give a smaller one when they stop reading or speaking. This delays the recovery in both stammering and normal speech; it is more noticeable on the normal speech curve because the curve is already low on the stammering plethysmograms. Less than half of the subjects show a decided attention drop; those who give it for reading also give it for every shift of attention; hence it can readily be distinguished as an attention drop.

Most of the stammerers who served as subjects had no fear of stammering in my presence and hence could not imagine themselves in embarrassing surroundings. Those who were able to imagine themselves in tight places were told that they would be called upon to introduce two persons or to buy a prescribed article at a store in a given number of seconds. During this period of suspense, Web averaged a drop of 11 mm. in an average of 14 seconds and Wi averaged a drop of 19 mm. in an average of 41 seconds. This shows that the fear of stammering causes marked vasoconstriction even when a stammerer is making no effort to speak.

It is useless for a stammerer to attempt to speak without hesitancy until peripheral vasoconstriction has ceased; hence he must wait at least twenty seconds before he commences to speak and should wait nearly a minute if he is a severe stammerer.

It will be seen that my results do not agree with Fletcher's. Fletcher found that in 90 per cent of his records a rise in the plethysmogram

began immediately after the initial attention drop and usually lasted until the end of the stammering period; I found that, with very few exceptions, the curve remained low from this attention drop to the end of the stammering period. Fletcher has published only a portion of two curves; the rise is not conclusive in either. In his plate D, the writing point which traced the pneumogram kept interfering with the writing point which traced the plethysmogram throughout the rise, lifting it forcibly in several places. In his plate E, there is no normal reference line to go by; the curve is cut from the middle of a period of stammering, and no normal curve is shown either before or after the subject stammered. The curve looks like some of mine that were traced immediately after the subject placed his finger in the plethysmograph when the air in the tubing was still warming enough to expand and cause the recording needle to rise steadily; I suspect that either the room or the air in Fletcher's connecting tubes was warming, causing the air in the tubes to expand and raise the writing point, and that the same rise or a greater one would have occurred during reverie. I frequently found gradual rises due to slow movements in the finger after attention drops, especially in shock; these gradual movement rises were always much steeper than the general rise going on at the same time, and ended in a gradual drop followed by a gradual return to the normal level in approximately that straight line, which, if produced, would pass through the point where the movement rise first began. If these gradual movement rises, due to involuntary, slow tightening or relaxing of the finger lasted for several seconds, it is reasonable to suppose that they would last longer in the arm and that Fletcher may have mistaken them for vasodilatation when he happened to stop the kymograph before the movement ceased and the curve began to fall. I used the Lehmann and the finger plethysmographs simultaneously on one subject and found that when the finger plethysmograph's recorder showed vasoconstriction, arm movements within the Lehmann plethysmograph often caused its recorder to register vasodilatation; the abrupt nature of these rises showed that they must be due to arm movement. All stammerers continually move their arms while stammering but seldom realize the minor movements. These movements affect the Lehmann plethysmograph much more than they affect the finger plethysmograph; it was for this reason that I discarded the former in favor of the latter. I think Fletcher expected a rise and was therefore prejudiced when tabulating his results. He may have discarded curves in which there was a fall, believing this fall was caused

by arm movements. Both Fletcher and I found that the distortions of the plethysmograms are correlated with the severity of the stammering; I found, however, that the amount of the general fall, rather than of the general rise, was correlated with the severity of the stammering.

My results also confirm Fletcher's statement that the curve showed no positive changes, and showed in certain cases a slight decline when the subjects were able to imagine themselves in situations where they would be likely to stammer; my subjects who not only imagined themselves in such situations, but actually lived over again such occasions, gave a marked drop (vasoconstriction), whereas those who were unable to live over again the embarrassing experience gave neither a rise nor a fall. The same vasomotor changes are to be expected whether a stammerer actually stammers or whether he lives over again a situation in which he stammered severely; my results showed vasoconstriction (never vasodilatation) in both cases; Fletcher looked for vasodilatation in both and was surprised to find vasoconstriction or else no vasomotor change when the stammerer imagined himself in a situation where he would be apt to stammer. As such a situation would be apt to be more free from arm movements than would a similar attack of stammering, I think that these results of Fletcher's are correct and that the stammering results are misinterpreted.

TABLE 7

Vasomotor changes in normal speakers accompanying shock brought on by short stimuli, long stimuli and compound stimuli

KIND OF STIMULI	NUMBER OF SUBJECTS	NUMBER OF STIMULI	FALL IN MILLIMETERS			t IN SECONDS			T IN SECONDS		
			Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
Short.....	7	67	26	10	1	70	18	2	100	39	7
Long.....	7	43	43	16	3	196	54	5	300	74	11
Compound.....	5	7	36	19	10	142	73	19	120	97	28

Table 7 shows the comparative vasomotor changes in shock produced by short stimuli, long stimuli and compound stimuli. All curves are those of seven normal speakers. Short stimuli include those listed in table 3. Long stimuli include reading silently several paragraphs from a very exciting novel or from a very horrible description such as *Murders in the Rue Morgue;* taking an unpleasant, unknown object, such as an artificial snake, out of a cloth bag; seeing material for a

TABLE 8

Vasomotor changes accompanying normal speech

SUBJECT	NUMBER OF PERIODS	AVERAGE LENGTH OF PERIODS	NUMBER WITH NO VASO-MOTOR CHANGE	NUMBER WITH UNQUESTIONABLE RISES	NUMBER WITH INITIAL ATTENTION DROP	NUMBER WITH FINAL ATTENTION DROP	INITIAL SHIFT OF ATTENTION						READING AND SPEAKING									FINAL SHIFT OF ATTENTION								
							Fall			t			Rise			t			T			Fall			t			T		
							Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum
K	20	69	9	10	2	1	3	2.5	2	22	17	12	11	3.8	2	108	55	25	68	33	17	3	3	3	16	16	16	27	27	27
E	4	57	0	4	0	0	0	0	0				10	7.0	3	60	53	38	48	36	22	0	0	0						
B	5	74	3	1	1	2	4	4.0	4	19	19	19	4	4.0	4	64	64	64	67	67	67	4	3	2	16	13	10	75	64	53
C	7	69	2	3	2	0	2	2.0	2	20	20	20	2	2.0	2	120	77	52	76	66	22	0	0	0						
G	3	63	0	3	0	1	0	0	0				7	5.7	5	43	38	34	60	36	17	7	7	7	23	23	23	45	45	45
L	7	119	2	1	4	3	15	13.0	11	95	37	12	4	4.0	4	110	110	110	100	100	100	19	11	6	12	7	4	44	40	35
M	8	58	3	2	0	3	0	0	0				3	2.5	2	23	18	13	24	22	20	10	6	4	14	10	4	52	35	13
N	3	66	1	0	2	0	4	3.0	2	13	8	5	0	0	0							0	0	0						
Q	5	95	0	0	5	0	7	4.8	3	60	35	15	0	0	0							0	0	0						
9	*62*	75	*20*	*24*	*16*	*10*	6	6.0	4	38	27	14	6	4.2	3	75	55	48	63	41	38	9	6.6	4	16	12	11	49	43	35

bonfire placed and being told to light it at a signal; and watching a mechanical frog set to go off several seconds after being placed. Compound stimuli consist of several short stimuli given simultaneously or in quick succession. It is evident that "t" will be lengthened in long stimuli and in compound stimuli given at intervals of a few seconds and that "T" will be lengthened when the time of maximum fall occurs near the beginning of a long stimulus or after the first part of a compound stimulus. This table shows that the maximum fall, "t" and "T" are greatest for compound stimuli and least for short stimuli. The maximum fall is 60 per cent greater for long stimuli than for short stimuli, and 90 per cent greater for compound stimuli than for short stimuli.

To ascertain whether the vasoconstriction which invariably accompanied stammering was due in part to reading or speaking per se, I had nine normal speakers read aloud and speak under the same conditions as the stammerers.

Table 8 shows the vasomotor changes of these nine normal speakers while they were reading aloud or speaking. These subjects read short stories which were free from excitement and gruesome descriptions so that emotions could not influence the vasomotor changes accompanying reading, and usually read in periods of from one to two minutes in order to give the vasoconstriction which sometimes accompanied the shift of attention to reading, time to eliminate itself half a minute or more before the end of the reading period.

"t" for "reading" was measured from the instant the subject began to read or speak to the moment when the maximum rise occurred on the plethysmogram. "t" for "final shift of attention" was measured from the end of the reading or speaking period to the time when the maximum fall occurred on the plethysmogram. "T" for "reading" included the final attention drop if there was one. The figures in the bottom line of this table denote the averages for their respective columns; the figures in italics, however, denote the totals for their respective columns.

Table 8 shows that there was no vasomotor change in 32 per cent of the reading and speaking periods; that there was an average rise of 4.2 mm. in 39 per cent of these periods; that there was an average attention drop of 6 mm. when the subject began to read or speak in 26 per cent of these periods; and that there was an average attention drop of 6.6 mm. when the subject stopped reading or speaking in 16 per cent of these periods. In addition to the twenty periods in which

there was no vasomotor change, there were three periods in which the only vasomotor change was an attention drop at the end of the reading or speaking period; hence the number of periods in which there was no vasomotor change practically equalled those in which there was vasodilatation. It is hard to classify under vasoconstriction, vasodilatation, and no vasomotor change, the periods in which there was an initial attention drop. If the maximum vasoconstriction occurred near the beginning of the period and the needle returned to its normal level as quickly as it did in the final attention drop for the same subject, it seemed logical to classify that period under either vasodilatation or no change; otherwise a period seemed to belong under vasoconstriction. As the needle usually returned to normal more quickly than it did in the final attention drop in the periods classified under vasodilatation and no change, and as it might have risen considerably had it not had to regain what was lost during the initial attention drop, I classified all such periods under vasodilatation. This classification gave 5 vasodilatations and 11 vasoconstrictions, which, added to the 24 unquestionable vasodilatations and the single unquestionable vasoconstriction, gave 71 per cent vasodilatations and 29 per cent vasoconstrictions where there was a vasomotor change. This agrees with the results obtained by Doctor Anderson, who found that mental work was accompanied by vasodilatation in 75 per cent of the periods of mental work, and that this proportion for reading was less than that for most other kinds of mental work. Vasodilatation predominated in some subjects, no vasomotor change in others. Attention drops occurred relatively frequently in some subjects and seldom if at all in others. Vasodilatation followed all of the initial attention drops, but a rise above normal occurred in but two periods; the needle returned to normal at or before the end of the reading or speaking period in about half of the periods. The initial attention drop was but two-fifths of that accompanying stammering and was reached twice as quickly in periods averaging approximately the same length, showing that this vasoconstriction was usually due to the shift of attention rather than to the reading. The maximum rise accompanying normal speech occurred at practically the same instant as the maximum fall occurred during stammering, i.e., 55 versus 53 seconds after the beginning of the period. The time of recovery averaged four-fifths of that in stammering, 41 versus 51 seconds, and was measured from the end of "t," which usually occurred several seconds before the end of the period, and not from the end of the period as in stammering.

It will be seen, therefore, that the vasoconstriction which always accompanied stammering was not caused by reading or speaking per se, but by stammering.

I watched the writing lever of the piston recorder whenever I gave a stimulus and noticed there was a latent period of a few seconds before it began to drop. I ran the kymograph at maximum speed for a few curves with a number of subjects to determine more accurately the length of this latent period. I found that this ranged from 1.6 second to 4.0 seconds, and that it averaged 3.4 seconds; this corresponds very closely with the latent period for smooth muscle. The average varied slightly in different subjects. The latent period was about the same in some subjects whether the stimulus was intense or slight; in other subjects it varied by as much as 2 seconds. The fall always began at the same point whether there was a temporary rise or not, and this rise never amounted to more than 2 mm. unless there was a violent movement of the finger. There was more apt to be a temporary rise when the subject jumped or when the stimulus was very intense. I feel sure this temporary rise was due to movement in the finger for it occurred too soon to be caused by vasodilatation. An unexpected stimulus causes one to tighten up more or less even if one does not actually jump; this tightening up is instantaneous but one does not relax at once. The jump itself, if there is one, does not last more than a fraction of a second and is registered as a vertical line on the curve; but there is a gradual readjustment after the jump which lasts for a few seconds. This lasts longer in the arm than in a single finger and explains the same temporary rise mentioned by so many other experimenters. Being convinced that this rise is due to movement and not to any vasomotor change, I have classed it under the general heading of fall, indicating vasoconstriction.

To determine whether the thought process was most impeded at the time when the stimulus was given or "t" seconds later when vasoconstriction in the periphery was at its maximum, I tied around the wrist of each subject's right hand a cord connected through pulleys with a writing lever which registered directly below the plethysmogram. I ruled three columns about one inch apart on a piece of paper and told the subject to add thirteen to the last number in the left column, then seventeen to the last number in the middle column, then twenty-one to the last number in the right column, then thirteen again, and so on; whenever his arm moved to the next column, it displaced the writing needle vertically. The horizontal lines on the record between

two vertical lines measure the number of seconds it took the subject to perform each addition. I gave several short, unexpected stimuli at intervals while the subject was performing this triple addition. A comparison of the plethysmogram with the curve below it showed that the abnormally slow solutions occurred when vasoconstriction was at its maximum, not when the stimulus was given.

SUMMARY

1. Shock and stammering are accompanied in every case by marked vasoconstriction. In a few cases this is preceded by a short, inconspicuous rise in the plethysmogram that is probably caused by arm movement.

2. Vasoconstriction does not begin until about three seconds after the stimulus is given.

3. Long stimuli are accompanied by greater vasoconstriction and slower recovery than are short stimuli.

4. Compound stimuli are accompanied by greater vasoconstriction and slower recovery than are either long or short single stimuli.

5. The more intense the stimulus and the more unexpected the stimulus, the greater is the vasoconstriction, the more rapid the vasoconstriction and the slower the recovery.

6. All the tables show marked individual differences even on the same record.

7. The greater the vasoconstriction, the more is verbal imagery impaired.

8. Those subjects who experience the greatest vasoconstriction during shock and stammering also require the longest time for recovery.

9. Marked peripheral vasoconstriction takes place more rapidly than does slight vasoconstriction.

10. Stammerers experience greater and more rapid vasoconstriction and slower recovery in shock than do normal speakers.

11. Stammerers as a class experience slightly greater vasoconstriction in shock than in stammering. The time of recovery is practically the same in both stammering and in shock. Those subjects in whom the fear of stammering is pronounced, however, experience greater vasoconstriction and slower recovery in stammering than in shock.

12. Vasoconstriction continues throughout the stammering interval; if any vasomotor change accompanies normal speech it is vasodilatation in a large majority of periods.

13. Fear of stammering with no attempt at speaking produces vasoconstriction in the periphery as does actual stammering.

14. Stammerers cannot speak without hesitancy during peripheral vasoconstriction.

15. All stammerers breathe abnormally while stammering. Every stammerer has a characteristic form of breathing while stammering.

THEORETICAL CONCLUSIONS

Stammering and shock are induced emotional disturbances accompanied by the same vasomotor changes. My experiments show that the vasomotor changes in stammerers as a class are of about the same magnitude and in the same direction in both stammering and shock. Unusually intense shock, such as the unexpected report of a pistol, is accompanied by greater vasomotor changes in the periphery than is stammering. Severe stammering, on the other hand, is accompanied in most subjects by greater vasoconstriction in the finger than is shock produced by any stimulus but the pistol. The time of recovery is about the same for stammerers as a class both in stammering and in shock, but is greater for most individuals in stammering than it is in shock produced by stimuli other than the pistol.

The experiments of Mosso and Shepard on trephined subjects reported in an earlier part of this monograph show that shock is always accompanied by vasoconstriction in the periphery and by vasodilatation in the brain. As my results for the periphery confirm those of these experimenters, and as I found approximately the same vasoconstriction in the periphery for stammering as I found for shock, there is every reason to suppose that stammering, like shock, is accompanied by vasodilatation in the brain and hence by cerebral congestion. I have been unable to find a trephined stammerer; as soon as I am able to locate a wounded stammering soldier who has been trephined, I hope to induce him to serve as a subject so that I can prove this supposition that stammering, like shock, is accompanied by cerebral congestion.

It is a well-known fact that fright, which is accompanied by cerebral congestion, may cause temporary partial paralysis of the speech mechanism and of other parts of the body. Persons lose their heads in panics and do inappropriate acts for which they censure themselves later.

Cerebral congestion affects the speech more easily than it affects any other human activity. Many persons have been struck dumb in intense fright. Many normal speakers repeat words or even stammer when greatly excited, and talk incoherently when mentally tired or confused; stammerers experience the greatest difficulty under these same conditions. Several of my normal subjects whose introspection is reliable reported that the words they were about to speak suddenly left their minds when the whistle blew and did not return for several seconds. Some of them still visualized the words and their minds seemed otherwise to work normally, yet the *sounds* of the words left them; hence they were unable to say the words. When vasoconstriction in their fingers ceased, the sound of the words reappeared and they were able to speak them. The same phenomenon occurs in stammering. The fear of stammering and the excessive effort put forth in forcing out difficult words cause cerebral congestion which causes the auditory images of the words the stammerer is about to speak to disappear as he attempts to utter them, just as the whistle banished the sound of the word the normal speaker wished to use and made of him a temporary stammerer. My plethysmograms show that one stammers only when there is vasoconstriction in the periphery and hence, as I have assumed, cerebral congestion. As the stammerer overcomes the impediment in his speech this peripheral vasoconstriction diminishes, and ceases when he is cured.

We see, then, that the fear of stammering and the forcing out of hard words which accompanies stammering cause vasoconstriction in the periphery and cerebral congestion. The latter blurs verbal imagery, especially auditory verbal imagery, and makes it impossible for the stammerer for the time being to recall a part or the whole of the word he wishes to speak at the moment he has to say it. As vowels are governed primarily by auditory imagery they are the stammerers' bugbears. The stammerer prolongs continuous consonants for seconds and repeats closed consonants over and over until able to recall the vowel that follows. The more the vowel is inhibited, the harder he forces out the preceding consonant, the more he thereby increases cerebral congestion, the more impossible it becomes to recall the vowel, and the worse he stammers. Many stammerers are thus led to believe that their trouble lies with the consonants they thus overdo and, by concentrating their mind on trying to force out these consonants, they keep the mind from recalling the vowel which fails to come promptly, and thus increase their stammering.

I have assumed, temporarily, that stammerers have normal verbal imagery when they are not actually stammering. I am conducting at the present time a comparative study of verbal imagery in stammerers and normal speakers which will either confirm or overthrow this assumption. This study will be published in a few months.

My experiments confirm Dr. C. S. Bluemel's theory that stammering is caused by transient auditory amnesia in the auditory speech center brought on by cerebral congestion.

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EXPLANATION OF FIGURES

Unless otherwise stated, the top line in figures 3 to 12 is the time line, the second line is the stimulus line, the third line is the thoracic pneumogram, and the bottom line is the plethysmogram.

Each notch in the time line in figure 3 represents two seconds. As the kymograph ran at approximately the same speed on all of these curves but those shown on figures 11 and 12, the time between any two points in figures 4 to 10 inclusive can be obtained approximately by measuring the distance between the two points and counting the number of notches in a like distance on figure 3 and multiplying by 2. (Allowance must be made for different reductions in reproduction). The exact time is recorded between two notches on the time line in all records, but all or part of this is sometimes missing from the part reproduced.

A notch on the stimulus line indicates when a stimulus was given or when a subject began or stopped reading or talking.

The top of the pneumogram indicates full lungs and the bottom empty lungs, unless otherwise stated.

The top of the plethysmogram indicates vasodilatation, the bottom vasoconstriction.

The following curves are all typical reactions; I have avoided reproducing extremes or abnormal curves. If any reader wishes to see my other curves, he is invited to examine those in my album at the Boston Stammerers' Institute.

Figure 3 shows a typical reaction of a normal speaker to shock. This curve was made by subject D on February 26, 1918. The tambour that recorded the thoracic breathing was inverted in this record so that the top of the pneumogram represents empty lungs and the bottom full lungs. At the notch marked *22*, I placed a mechanical frog near the subject. At the next notch, about a quarter of an inch to the right, this frog jumped 4 feet into the air without the slightest warning. At *23*, the subject read silently a dull book until a point well beyond the part reproduced; this occupied the subject's attention and thus prevented arm movements. Note the fall of the plethysmogram when the frog was placed, the temporary rise when the frog jumped (due here to movement when the subject jumped), the decided drop following this brief rise, and the much slower return to normal. Other curves show that recovery is the same whether the subject is in a state of reverie or is reading silently. The straight line tangent to the plethysmogram at its two ends is the path that would have been traced had no stimulus been given.

Figure 4 shows two typical reactions of a stammerer to shock. This curve was made by subject Wi on August 1, 1918, a few minutes before the curve reproduced in figure 5. At *W*, a shrill whistle was blown without warning and at *Y*, the operator gave a loud, sudden yell. The time line shows that an interval of a minute and a half elapsed from the notch to the right of *W* to the notch to the left of *Y*. The straight lines on the right hand half of the plethysmogram show how "Fall," "t" and "T" are measured; the line tangent to the plethysmogram at its two ends shows the path that would have been traced had no stimulus been given, the vertical line represents the maximum fall and cuts the stimulus line at a point "t" seconds from the notch where the yell was given and "T" seconds from the point of tangency at the extreme right of the reproduction. Note that the drop for the whistle is much greater than that for the yell.

Figure 5 is a typical stammering curve made by subject Wi a few minutes after the curve shown in figure 4. At the left hand stimulus notch, this stammerer was told that he would be called upon to introduce two persons in fifteen seconds. At the longer notch, about half an inch to the right, he began the introduction, stammering considerably; at the next notch, a quarter of an inch to the right, he finished speaking. The long notch in the time line has no significance. Note that the curve fell rapidly soon after the subject was told that he would be asked to introduce two persons and remained low throughout this period of warning, that it fell nearly as much below this point soon after the subject began to stammer and remained low throughout the stammering interval and for a few seconds after stammering ceased, and that it returned much more gradually to normal. The room was cooling down somewhat, hence the curve fell gradually throughout the record.

Figure 6 is a typical curve of a long period of stammering made by Wi on the same day as that reproduced in figure 5. At the notch below *17*, the subject was told to check a trunk to Portland, Maine, in forty-five seconds. At *18*, he began to speak to a baggage master whom he saw clearly in his imagination and I kept him stammering to the first long notch on the time line by asking him unexpected questions about his trunk. The short notches close together on the time line indicate when he was stammering most severely. The two long notches at the right with *1 m* between them indicate a period of one minute. The straight

line below the pneumogram represents the path that would have been traced if the subject had not been disturbed. Note that the curve fell rapidly soon after the stammerer was told he would be called upon to check his trunk in forty-five seconds and remained low throughout both this interval and the stammering which lasted about a hundred seconds. There was a gradual arm movement during a part of the period of recovery.

Figure 7 is a typical curve of a stammerer reading without being told in advance that he is to be called upon to read. It was made by Bo on April 23, 1918. At the notch below *3*, he began to read and at *4* he stopped reading. Note the steady fall throughout the reading period and the steady but more gradual rise to normal, beginning with a slight finger movement at the close of the reading period. There is another gradual finger movement near the end of the period of recovery. The pneumogram is inverted in this record.

Figure 8 is a typical curve made by a cured stammerer reading without hesitancy. It was made by Bo on November 26, 1918, after he had been taught how to read without hesitancy. The subject read without stammering for one minute between the single and double notches on the stimulus line. There is a distinct breathing wave in the plethysmogram (due to movement). There was a gradual movement of the finger at the end of the reading period. The curve rose very slowly throughout the record because the air within the tube was warming up and expanding. The breathing appears very much deeper on this record than on the others because a much more sensitive pneumograph was used; this pneumogram is not inverted. Note that the plethysmogram did not fall as it did in figure 7, when the same subject stammered severely while reading a similar passage from the same book.

Figure 9 is a typical curve of a normal speaker reading, showing an attention drop. At *5*, the subject began to read; at *6*, he stopped reading. The time notches above *5* and *6* indicate that he read for exactly one minute. After the attention drop at the beginning of the reading, there was a slow rise to normal with occasional gradual arm movements. The curve rose gradually throughout this experiment, as the air in the tube was growing warmer.

Figure 10 is a typical curve of a normal speaker reading, showing no attention drop. He read for about one minute, beginning at the left hand notch and stopping at *6*. The curve is as regular as that in reverie preceding and following it. The subject's finger moved slightly while he was reading. The engraver omitted the time line which happened not to be needed.

Figure 11 is a typical shock curve traced when the kymograph was running at maximum speed to show the latent period of vasoconstriction. It was made on November 18, 1918, by the same normal speaker who made the curve reproduced in figure 9. At the extreme left hand notch on the stimulus line, I yelled suddenly. The time notches in the right hand half of the record were made at intervals of ten seconds. Note the slight rise due to arm movement the instant I yelled, the distance from this point to the point where vasoconstriction began, the rapid vasoconstriction and the gradual arm movement at the beginning of the slow rise to normal which is reached at the very right hand end of the reproduction. Reference lines made at the right hand end of all four curves show that the four recording needles were working nearly in a straight line. The breathing appeared more shallow than that in the other records because the less sensitive Verdin pneumograph was used.

Figure 12 contains a number of pneumograms taken at maximum speed. The left hand half of the one at the top is that of a normal speaker reading. The right hand half is a typical pneumogram of a cured stammerer reading without hesitancy. The pneumogram just below is that of a stammerer who habitually spoke on empty lungs. The middle one is that of a stammerer who habitually spoke on full lungs. The two near the bottom are those of stammerers who spoke now on full lungs and now on empty lungs; note their irregularity.

Fig. 3. Three-fourths natural size

Fig. 4. Three-fifths natural size

Fig. 5. Two-thirds natural size

Fig. 6. Three-fifths natural size

Fig. 7. Four-fifths natural size

Fig. 8. Three-fifths natural size

Fig. 9. Three-fourths natural size

Fig. 10. Three-fourths natural size

Fig. 11. One-half natural size

Fig. 12. One-third natural size

A NOTE ON INTRAVASCULAR FAT IN RELATION TO THE EXPERIMENTAL STUDY OF FAT EMBOLISM IN "SHELL SHOCK"

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The prominence recently given to the hypothesis that "explosive shock" is due to fat embolism has led to the histological examination for intravascular fat in the brains and other organs of dogs subjected to concussion by Dr. D. R. Hooker, for the Committee of Physiology of the National Research Council, in his studies on the physiological effects of air concussion. These observations have been controlled by the examination for fat in the blood vessels of normal laboratory animals.

METHODS

Material preserved in formaldehyde and fresh tissues have been used in this study. Free-hand sections made with a razor blade or frozen sections, pieces of the meninges with their vessels and pieces of the choroid plexuses of the brain have been stained with Sudan III, Scharlach R and with osmic acid. Staining with Sudan III has been employed most frequently and as follows:

Stain 1 to 3 minutes in
Sudan III, saturated solution in 70 per cent alcohol..... 100 cc.
Sodium hydroxide (sticks)..... 1 gm.
(filter after sodium hydroxide has dissolved)
Rinse in 60 per cent alcohol.
Pass directly to glycerine.
Note: Sudan III (Grübler) and Aniline Red, Sudan III oil as sold by Eimer & Amend, have been used.

After staining and when cleared in glycerine, all sections or pieces of tissue were examined with a binocular microscope with a 32 mm. objective and low-power oculars. For closer observation, some prepa-

rations were mounted on slides under cover glasses in glycerine and examined with the compound microscope.

In order to demonstrate accurately the intravascular position of fat droplets in the choroid plexuses and in other small cerebral vessels with collapsed walls, India ink has been injected through the arteries of the head prior to fixation. Preparations of the choroid plexuses stained with Sudan III, after this injection with India ink and fixation with formaldehyde, show clearly the position of the fat droplets with reference to the ink particles contained in the vessels (see dog 13).

To test the accuracy of the staining with Sudan III, osmic acid has been used. Sections or pieces of tissue containing vessels holding droplets stained with Sudan III have been treated with 1 per cent osmic acid while under observation with the microscope. The characteristic blackening of the droplets begins at once, replacing or superimposed upon the clear orange color which follows treatment with Sudan III.

Examination of the liver, dura and choroid plexus, fresh, without previous fixation (dog 12), and observations on the vascular content of the liver in dog 13 have been made to control the observations on material fixed in formaldehyde.

Dogs. Observations on intravascular fat have been made on ten dogs, five control animals, four exposed to and shocked by air concussion due to gun-fire and one subjected to prolonged anesthesia. The histories and findings in these dogs are as follows:

Dog 1. Adult male. Subjected to air concussion and shocked. Dead ten and a half hours after exposure and immediately preserved with formaldehyde. Central nervous system saved.

Vessels of the dura and arachnoid and those of the choroid plexus of the third ventricle show intravascular fat.

Dog 2. Adult male. Subjected to air concussion and shocked. Dead four hours after exposure and immediately injected with formaldehyde. Central nervous system saved.

Fat droplets present in vessels of the choroid plexus of the third and lateral ventricles and in vessels of the dura.

Dog 7. Adult male. Subjected to air concussion and shocked. Dead two hours after exposure. Central nervous system fixed by injection with formaldehyde.

Fat droplets present in vessels of the choroid plexus of the fourth and lateral ventricles and in the venous capillary expansions of the dura.

Dog. 10. Adult male. Following morphia and the destruction of ear drums and ossicles under ether, subjected to air concussion with abdomen covered with cotton pad and plaster bandage. Shocked. Dead thirty minutes after exposure. Central nervous system and viscera preserved in formaldehyde.

Fat droplets present in the vessels of the choroid plexus of the fourth ventricle and in vessels of the dura.

Smaller vessels of the lungs and kidneys contain occasional droplets. No fat found in vessels of the spleen, liver, pancreas, cardiac muscle or suprarenal.

Dog A. Adult male. One hour after the administration of one grain of morphia, ether given and continued through tracheal cannula for two and a half hours, when the animal was bled to death. Blocks of the brain were removed fresh and the remainder of the central nervous system preserved immediately in formaldehyde.

Fat droplets present in the vessels of the choroid plexus of the third and fourth ventricles and in vessels of the dura and corpus striatum.

Dog B. Adult male (control for A). Killed by bleeding following local anesthetic. Central nervous system fixed in formaldehyde.

Vessels of the dura and of the choroid plexus of the third and lateral ventricles contain fat droplets.

Dog 9. Adult male (control for no. 7). Killed by intravenous injection of ether and immediately injected with formaldehyde. Central nervous system saved.

Droplets of fat found in vessels of all of the choroid plexuses, the dura and arachnoid and in vessels within the substance of the corpus striatum.

Dog 11. Young adult male (control). Killed with illuminating gas. Central nervous system and viscera immediately fixed by immersion in formaldehyde.

Fat droplets present in all choroid plexuses, in vessels of the dura and arachnoid and in the posterior inferior cerebellar artery.

Vessels of the lungs, kidney and liver contain fat droplets. None demonstrated in vessels of the spleen, pancreas, suprarenal or cardiac muscle.

Dog 12. Adult male (control). Killed with illuminating gas. Central nervous system and viscera fixed by immersion in formaldehyde, except a portion of the liver, a piece of dura and a part of the choroid plexus of the fourth ventricle, which were examined fresh.

When examined without previous fixation, vessels of the liver, of the dura and of the choroid plexus of the fourth ventricle contained fat droplets stainable with Sudan III and with osmic acid.

After fixation with formaldehyde, fat was demonstrated in vessels of the dura, arachnoid and all of the choroid plexuses.

After fixation with formaldehyde, fat droplets were observed in vessels of the lung, liver, kidney and suprarenal. None was seen in vessels of the spleen or cardiac muscle.

Dog 13. Adult female (control). Killed with illuminating gas. Liver removed entire with blood contained and immersed in formaldehyde. Both common carotid arteries isolated and warm Ringer's solution passed through the head until the solution ran clear. Tourniquet applied to neck below the site of carotid exposure. Solution of India ink injected in the right carotid until it ran from the left carotid. Left carotid then clamped and a little more ink injected with syringe into the right carotid. Both carotids then tied, brain exposed and head fixed by immersion in formaldehyde. The vessels of the choroid plexuses and of the meninges and brain were incompletely injected with the ink.

Fat droplets demonstrated, after staining with Sudan III, in vessels of the dura, arachnoid and in vessels of all choroid plexuses.

In the brain of this dog, following injection with India ink and staining with Sudan III, particles of ink and fat droplets were sometimes found together in the blood vessels. Many of the capillary loops of the choroid plexuses, incompletely injected with the ink, were found to be occupied by fat droplets. The capillary loops of the choroid plexuses, well injected with the ink, were usually found free from fat droplets. In the flat venous capillary expansions found in the dura, particles of ink and fat droplets, more or less finely divided, were found constantly together.

Pieces from the surface of the liver, immersed in formaldehyde as above, were taken 3, 5, 24 and 72 hours after the beginning of fixation and examined for fat. Intravascular droplets were demonstrated in all. At the end of 24 and 72 hours, after the beginning of fixation, pieces of liver were taken from the surface and from 5 and 10 mm. beneath the surface and examined for fat. All pieces showed intravascular fat droplets.

In the routine examination of brains for intravascular fat the choroid plexuses and dura were chosen as parts containing a great number of capillary vessels. They proved to be easily handled by the methods employed and easily studied when spread and observed with a binocular microscope. Sections of the brain itself were made in some cases but the difficulty of handling these sections and clearing them in glycerine for accurate observation, the necessity of thinness and the relatively small number of capillaries available for study in even a considerable number, made their use impractical. The choroid plexuses were removed and stained entire and when spread in glycerine after clearing, presented a great number of capillary vessels covered only by the transparent ependyma. In several cases fat droplets were found in all the choroid plexuses taken from a single brain but usually such droplets were confined almost wholly to one plexus. The choroid plexus of the third ventricle, in our observations on dogs, has held by far the greatest number of fat droplets; in several cases almost a whole half of the plexus contained droplets, practically every one of the many capillary loops being filled with them.

Pieces of the dura were found to be quite easily handled and cleared. When cleared and examined with the high power objective of a compound miscroscope, many of the capillaries and the capillary expansions between the arterial and venous capillaries were found to contain blood and, with the blood, droplets of fat. Injection with formaldehyde or Ringer's solution frees the larger dural vessels of blood but the contents of most of the capillaries and their expansions seem to be incompletely removed by such injection.

In the dog, as well as in the rabbit and cat, intravascular fat has been found in the form of single droplets or in groups of several droplets. These droplets vary in size from those easily seen with the binocular microscope to those just visible with the low-power objective of the compound microscope. In the cerebral vessels single droplets only have been observed occurring singly in the larger vessels and often in rows in the capillary loops of the choroid plexuses. In the choroid plexuses large droplets have been quite constantly found, droplets whose diameter is usually greater, when observed after the death of the animal, than that of the capillary containing them. In the venous capillary expansions of the dura, on the other hand, most of the droplets observed have been small, little larger than the red blood corpuscles occurring in the vessels with them. There is evidence in some preparations of the dura that several of these small droplets may coalesce to form larger ones. In sections of the lung, fat droplets have been frequently found pressed out in passing through a small capillary loop, so that they present after fixation a bent cylindrical form completely filling the small capillary.

Careful examination of the above findings in dogs shows that fat has been demonstrated in the blood vessels of all examined, no matter whether the individual has been shocked by air concussion or not. No quantitative or qualitative differences have been observed between the fat found in the vessels of the shocked animals and the intravascular fat of normal controls. Exact quantitative estimation of fat present in the vessels is of course impossible with the methods used, but its incidence in various parts of the vascular system subjected to examination in the routine study of ten dogs would indicate roughly a considerable quantity in all. If there is any difference between the amount present in the vessels of shocked and control animals, the above observations would lead one to believe that there is more in the control dogs than in those shocked.

It is interesting to note also that the fat present in the vessels examined does not seem to have been affected by the cause of death; animals dying from shock, those killed by bleeding, with ether or illuminating gas, showed no essential differences in the fat present. Death by bleeding certainly would remove most of the fat contained in the vessels completely emptied, but it is evident that death by bleeding does not remove the fat droplets contained in the small vessels examined. Further, in dog 13, where the intravascular position of the droplets contained in the capillary vessels of the choroid plexuses and

in the capillary venous expansions of the dura was established with certainty, the common carotids were cut, the animal allowed to bleed, the branches of the common carotid then being washed clean with warm Ringer's solution before the injection of India ink. Notwithstanding this bleeding and washing, fat droplets were still found along with the injected ink particles It is evident then that bleeding or washing out the vessels of the head after bleeding fails to remove completely the fat droplets found in the capillary vessels examined. In the animals injected with formaldehyde for purposes of fixation, it follows that the fat droplets under observation have been little if at all affected by the injection.

It might be considered possible that the fat droplets observed in the vessels after fixation with formaldehyde were changed in size, aggregation and staining reaction by post-mortem factors and fixation. To control such possibilities, sections of the liver and pieces of the dura and choroid plexus of the fourth ventricle were stained with Sudan III and osmic acid, fresh, as soon after the death of the animal as they could be removed (see dog 12). Fat droplets, similar in size, location and staining reaction to those found in the same animal after fixation with formaldehyde were observed in this fresh tissue. Further, that the length of time of fixation with formaldehyde or its penetration, within the limits of our observations, do not change the amount or character of the fat recognizable histologically in vessels of the liver, has been shown by the observations on dog 13 above. It seems probable then that fixation in formaldehyde has not altered the frequency or character of the fat droplets demonstrated histologically in the present observations.

Rabbits. Four large normal rabbits, bled to death for the production of normal serum, were used in these observations. These rabbits averaged about 2,500 gm. in weight and as blood was withdrawn, Locke's solution was supplied intravenously, each rabbit yielding approximately 300 cc. of blood after receiving about 150 cc. of Locke's solution. During this procedure the blood vessels of these rabbits must have been well irrigated so that the amount of intravascular fat found may not represent what was present before the bleeding. It seems probable, however, from our findings in the brain of dog 13 following bleeding and irrigation with warm Ringer's solution, that the amount of fat in the cerebral vessels examined in these rabbits was little affected. The amount present in the other organs examined may have been reduced. The brains and lungs of all four of these

rabbits were preserved in formaldehyde. Other viscera from one rabbit were also saved in formaldehyde.

Fat droplets were found in the cerebral vessels of all four rabbits, particularly in the dural venous capillary expansions and in the vessels of the choroid plexuses. Thick sections were made from blocks taken at random, one from each pair of lungs. Fat was demonstrated in the capillary vessels in two of the four. In the other viscera saved from one rabbit, the kidney alone showed intravascular fat after a routine examination of two sections from each organ. These fat droplets were observed in the cortical vessels of the kidney.

Cats. The cats first examined, series 1, had been used for other experiments in this laboratory. Some died or were sacrificed with acute meningeal infection or following intravenous and subarachnoid injections of various sorts. These cats had been fed on a comparatively fat-free diet and following the experiments to which they were subjected were not, at the time of death, in a state of good nutrition. It will be seen from series 1 below, that intravascular fat was found in none of the choroid plexuses taken from such animals and that the capillary vessels of only five of sixteen pairs of lungs examined were shown to contain fat.

Series 1. Cats fed for some time on a comparatively fat-free diet, some with acute meningeal infection, others subjected to intravenous and subarachnoid injections.

Choroid plexuses of brain. Of ten examined, none showed intravascular fat droplets.

Lung (one block from each pair of lungs). Of sixteen examined, five showed a few fat droplets in capillary vessels (these droplets fewer and more scattered than those observed in pulmonary vessels of the dog or rabbit).

In view of the above, an attempt was made to feed cats a diet rich in fat, preparatory to examination of intravascular fat. The first series of two kittens was complicated by the appearance of distemper which shortened the period of feeding, but in spite of this the kittens showed relatively more intravascular fat than was observed in the cats in series 1.

Series 2. Two kittens fed cream for three days; both contracted distemper, one died in cage. At autopsy, evidence of meningitis in both.

Choroid plexuses of brain. Three droplets found at tip of one of the capillary loops in the choroid plexus of the third ventricle in one kitten.

Dura. Finely divided fat droplets present in the smaller veins and venous capillary expansions in both.

Lung (one block from each pair of lungs). Several groups of free intravascular fat droplets were present, in addition to many intracellular granules.

Kidney (one block from one kidney of each kitten). Fat droplets present in vessels of the cortex in addition to intracellular fat of the cortical tubules in both kittens.

Finally, three healthy, well-nourished, normal adult cats were taken and fed a diet rich in fat for five days before killing. A fourth, normal healthy male, was killed as received without feeding and included in this series. While the amount of fat demonstrated histologically in the blood vessels of these cats seems relatively less than that found in dogs and rabbits, still it is greater than that observed in the poorly nourished cats of series 1. Further, in this series thirteen organs were examined from each of two fat-fed cats and one normal adult cat killed without feeding. In one fat-fed cat, intravascular fat droplets were found in eight of these thirteen organs and in the other fat-fed cat, in six. In thirteen organs taken from the cat killed without feeding, fat droplets were demonstrated in only four.

Series 3. Normal adult cats—three which were fed a rich fat diet for five days, the fourth, a healthy normal male, killed as received without feeding.

Dura. Fat droplets present in dural vessels of the three fat-fed cats; dura of the fourth not examined.

Choroid plexuses of brain. A single droplet observed in vessels of the choroid plexus of two of the fat-fed cats.

Arachnoidal vessels. Fat droplets present in arachnoidal vessels in one fat-fed cat and in the fourth, killed without feeding.

Lung (one block from each pair of lungs). Fat droplets found in pulmonary vessels of the cat killed without feeding—lungs from three cats examined.

Kidney (three cats, two of which were fat-fed). Fat droplets present in cortical vessels of all kidneys examined.

Sections from eight other organs examined in three cats.

The above observations may indicate that the feeding of a rich fat diet in cats may increase the number of intravascular fat droplets, recognizable histologically.

Of the three fat-fed cats in series 3, two were killed with illuminating gas, the third with ether. No essential differences in the incidence or amount of the intravascular fat was observed depending on the means of death.

All the observations on cats seem to show that there is present in this animal relatively less intravascular fat, recognizable histologically in the organs examined, than is found in the dog or rabbit. This is

most noticeable in the choroid plexuses where in the dog and rabbit, so far as our observations go, it seems to occur constantly in some part of the choroid plexus.

SUMMARY

1. Free fat droplets, stainable with Sudan III, Scharlach R or osmic acid, are found in the blood vessels of the brain, lungs and other organs of the dog and rabbit in considerable number. In the cat, although present, this intravascular fat seems to occur in smaller amount than in the dog or rabbit.

2. Observations on dogs indicate that there are no quantitative or qualitative differences between the fat, recognized histologically, in the vessels of normal control animals and that demonstrated in dogs subjected to concussion or prolonged anesthesia.

3. In cats it seems probable that a rich fat diet increases the incidence and amount of intravascular fat demonstrated histologically.

ON THE ACTION OF CERTAIN SUBSTANCES ON OXYGEN CONSUMPTION

II. Action of Potassium Cyanide on Planaria

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INTRODUCTION AND PRESENTATION OF LITERATURE

In the first paper of this series (1) I believed that I had shown in a sufficiently convincing manner that potassium cyanide decreases oxygen consumption. Sponges were selected as the most favorable organisms for such experiments since in such simple animals the action of drugs like cyanide must be a purely cellular one. As the results were definite and striking and in complete accord with the prevailing opinion regarding the mode of action of cyanides on protoplasm, it did not occur to me that any further experiments with this particular substance were necessary. Subsequently, however, Lund (2) published a paper in which he stated that he was unable to find any decrease in the rate of oxygen consumption in Paramecium in the presence of cyanide. In this paper, the considerable number of investigations now at hand regarding the physiological and pharmacological action of the cyanides were very inadequately considered and no explanation was given as to why Paramecium should differ from other organisms and cells in this respect. The fact is that all investigations, except those of Lund, in which the action of cyanides upon the rate of oxygen consumption has been directly measured have demonstrated a striking decrease in rate of oxidation. In view of this evidence, the only conclusion which is warranted from Lund's experiments is either that Paramecium is an exception which requires special explanation or that the experimental method employed by him is somewhere at fault.

Since, however, Lund seems to think that these experiments entitle him to raise objections to the conclusions which have been drawn by Child (3) and other members of this laboratory from researches in

which potassium cyanide (but also other reagents) has been employed, it has become necessary for us to determine directly the action of potassium cyanide upon the oxygen consumption of *Planaria dorotocephala*, the animal which has been extensively used in the experiments referred to. I therefore undertook to measure the rate of oxygen consumption of Planaria in normal and cyanide-containing water. The results of this investigation are reported in the present paper. I may say at once that in my experiments, numbering nearly seventy-five, with concentrations of potassium cyanide ranging from 1/1000 to 1/200,000 mol., I found without exception a decrease in the rate of oxygen consumption in the presence of cyanide. In the stronger concentrations, the amount of oxygen consumed fell to 10 to 20 per cent of the normal value; with increasing dilution of the cyanide solution, the amount of decrease gradually diminishes. In every case complete recovery occurred, the oxygen consumption returning to the former rate when the animals were replaced in water and thoroughly washed.

Before presenting my experiments in detail, I wish to review the literature of this matter since it has been largely ignored by those who would like to think that cyanide does not decrease the oxygen consumption of protoplasm. This literature may conveniently be considered under four heads: the chemical structure and properties of the cyanides; their effect upon enzyme and other catalytic actions; their physiological effects; and their specific action upon the respiration of living material.

The structure of potassium cyanide was elucidated through the work of the late Prof. J. U. Nef. As is well known, Nef believed that many organic reactions involve first the dissociation of the reacting substances into radicals containing unsaturated carbon atoms, and then the union of such radicals with other groups by way of these unsaturated atoms. Starting from the fact that only those organic compounds which contain unsaturated carbon atoms ("double" and "triple" bonded carbon) are markedly reactive, Nef concluded that organic reactions in general are due to the presence of such unsaturated atoms, expecially bivalent carbon. Methylene, $H-\underset{\displaystyle H}{\overset{\displaystyle |}{C}}=$, is the simplest compound containing bivalent carbon, but this cannot exist, according to Nef, because its bivalent carbon atom is so extraordinarily reactive that it promptly unites with another molecule of itself or with any

other available group. Nef suspected that the inorganic and organic cyanides (nitrils) are really substitution products of methylene, having the general formula, R—N=C=, and that therefore they must react in a manner identical with the olefines, substances known to contain unsaturated carbon atoms and chemically characterized by their ability to form additive compounds. He found (4) that this was indeed the case, and the cyanides will form additive compounds with great avidity with halogens, halogen acids, sulphur, hydrogen sulphide, phosgene, ammonia, acetyl and benzoyl chlorides, alcohols, aldehydes, primary amines, etc., and polymerize readily. They also add oxygen with great ease, forming R—N=C=O (this may go over into more complex oxygenated compounds). In short, the cyanides[1] are extremely reactive substances; they rank as the most powerful reducing agents known; they interfere with oxidations and unite readily with other groups, particularly unsaturated ones (5), wherever there is an opportunity for them to do so. It should be noted that such unions commonly interfere with the course of the reaction, when cyanide is added to reacting substances, as appears to be always the case in living materials, but they need not necessarily do so since the compound so formed may conceivably be more favorable for the reaction than the original compounds, through greater solubility, for instance.

The reducing power of the cyanides can be readily demonstrated. If potassium cyanide is added to a solution of potassium permanganate, the purple color disappears, a reduction of the septivalent Mn ion to the bivalent condition; and similarly potassium bichromate turns green, hexavalent Cr. having been reduced to trivalent Cr. This capacity of the cyanides for taking up positive charges enables them to keep substances in contact with them in a reduced condition, or conversely, to prevent them from undergoing oxidation, since oxidation consists in the addition of positive charges. Geppert (6), one of the early workers on the action of cyanide on animals, attributes its power to decrease oxygen consumption to this property, and cites several well known oxidations with which potassium cyanide interferes. One

[1] As Nef points out, substances containing the N=C= radical should properly be designated as isocyanides or isonitrils, since there is a group of substances containing the C≡N radical for which the name cyanide or nitril should be retained. In fact, these substances are tautomeric, a solution of any of them containing both radicals, although there is very little C≡N present in solutions of potassium cyanide. However, as this terminology has not come into general usage, it is not employed here.

of the most striking examples of the power of cyanides to prevent oxidation reactions is that discovered by Mathews and Walker (7), who found that the spontaneous oxidation of cysteine to cystine is retarded by small amounts of potassium cyanide and other isocyanides (nitrils) and prevented by larger amounts. The authors explain the inhibition as due to the union of cyanide with cysteine at the point where oxidation would otherwise occur; this explanation is in accord with the properties of the cyanides as already explained. Curtman and Kaufman (8) have recently called attention to the interference of potassium cyanide with the whole group of oxidations commonly used in quantitative analysis in which iodine is set free through the oxidation of an iodide. Whatever the oxidizing agent employed, some of the liberated cyanide promptly disappears when cyanide is present. Anyone can readily observe for himself the fading of the iodine color in such solutions upon the addition of iodine. Since the Winkler method for the determination of the oxygen content of water depends upon such an oxidation of iodide to iodine, it is quite obvious that it is chemically impossible to determine absolutely the oxygen content of cyanide-containing water by this method. One has to depend upon relative results, or remove the cyanide.

The foregoing examples suffice to demonstrate the inhibiting effect of cyanides upon oxidative reactions *in vitro*, and there is no reason to assume that their action *in vivo* would not be of the same character.

Cyanides also interfere with the activity of organized and unorganized catalyzers. They greatly retard and even completely prevent the catalytic splitting of hydrogen peroxide by finely divided platinum, silver and other heavy metals. This catalysis is commonly believed to be due to the increased adsorptive surface provided by such finely divided metals. This explanation appears to me to be inadequate, for the following reasons. Loevenhart (9) and Kastle and Loevenhart (10) found that cyanide and a number of other substances which retard this catalysis produce their effect by forming a film of insoluble salt around the metal particles. If the effect of the metal particles is solely physical, why should the presence of this film retard the action? On the other hand, the same substances which inhibit catalysis by platinum and silver markedly accelerate the same catalysis when copper and iron are used and the cause of this acceleration is the union of the accelerating substances with the metals to form soluble salts. If the catalysis is due to adsorption on the metal particles, the change of these metal particles into soluble salts passing into true

solution ought to interfere with the catalysis, but as a matter of fact it markedly accelerates it. The catalysis therefore is probably primarily a chemical reaction, whose speed depends on the solubility of the substances present. The mechanism of many familiar reactions in which catalyzers are necessary is now known to be chemical; for instance, the formation of esters from fatty acids and alcohols proceeds very slowly unless acid is present. The acid produces its accelerating effect by uniting with the substances present to form a complex salt which decomposes to yield the ester and the original amount of acid very much faster than the ester would be formed in the absence of acid.

Loevenhart (9) and Kastle and Loevenhart (11) state that all substances which catalyze hydrogen peroxide also bring about oxidations, such as the bluing of tincture of guaiacum, oxidation of phenolphthalin to phenolphthalein, etc. Potassium cyanide and other substances which inhibit the catalysis of hydrogen peroxide by certain metals also inhibit these oxidations; but with other metals, such as copper, cyanide causes an acceleration of both the catalysis and the oxidation reactions. The same authors found that, although hydrogen peroxide oxidizes guaiacum to only a slight extent, other peroxides like benzoyl peroxide, lead peroxide, silver oxide, manganese dioxide, readily accomplish this oxidation. Now, the bluing of guaiacum by certain of these peroxides, the first three mentioned, is markedly retarded by cyanide; but in the case of manganese, the oxidation is accelerated.

The conclusion which I wish to draw from these facts is, I think, a significant one,—namely, that the action of cyanide or any other substance upon a given chemical process depends primarily on the nature of the compound formed by the cyanide with the reacting substances. If this compound is less active than the other reacting substances, through insolubility, slight dissociating power or other causes, the reaction will be retarded or even completely inhibited. If the compound formed is more active, then the reaction will be accelerated. This point of view should replace the old dogmatic idea that cyanide is "a specific inhibitor" of oxidations or any other reactions. *It merely happens* that in the case of living, organized materials, the compounds formed with cyanide are nearly always much less reactive than the original substances, so that biochemical processes are in general retarded.

The retarding effect of the cyanides upon certain organized catalyzers, that is, enzymes, has long been known. According to Fiechter (12), the enzymatic power of intact bacteria, yeasts and fungi is completely

inhibited by hydrocyanic acid. This author and also Schönbein (13) found that yeast is entirely unable to split glucose in the presence of HNC; the effect is completely reversible, the fermentation proceeding as soon as the hydrocyanic acid evaporates. These retarding effects of the cyanides upon enzyme-containing cells are probably to be regarded as due to inhibition of the general metabolic processes in such cells rather than as actions upon the enzymes themselves.

It is significant, in view of the strong reducing power of the cyanides, that they particularly inhibit the activities of those enzymes associated with oxidation processes in cells; that is, the catalases, oxidases, peroxidases and reductases. Schönbein (13) discovered that practically all tissues, both plant and animal, have the property of splitting hydrogen peroxide into water and oxygen. Loew (14) showed that this property was due to a specific enzyme (or enzymes) to which he gave the name catalase. The recent studies of Burge (15) have shown that in animals catalase is closely associated with oxidation processes, since factors which are known to increase the respiratory exchange, as muscular activity, feeding of thyroid, etc., also increase the catalase content of organs, while factors which decrease respiration likewise decrease the catalase content. The same has been demonstrated for plants by Appleman (16). He found that various treatments which increase the respiratory exchange in potato tubers, such as soaking in ethyl bromide, changing from a lower to a higher temperature, greening in light, sprouting, also augment the catalase content of tubers. The activity of catalase, whether in its normal relations in intact cells, such as yeast cells and red blood corpuscles, or in extracts, is completely inhibited by cyanides, so, that not a trace of oxygen is evolved from the solutions. The effect is quite reversible, the usual amount of oxygen being released when the cyanide volatilizes. This action of cyanide on catalase was discovered by Schönbein (13) and has been repeatedly confirmed since by Schär (17) and Jacobson (18), for example, who used not only hydrocyanic acid but also some substituted cyanides with the same result. Moore and Willaman (19) investigated the action of HNC on the oxidases and catalases of plants. The oxidases from dried tomato leaves or tomato juice are reversibly inhibited by hydrocyanic acid; and oxidases obtained from plants after fumigation with HNC are less active than those from normal plants. Fumigated plants carry on less photosynthesis than normal ones and they fail to use the starch which they produce; this last effect was shown to be due to an inhibition of the consumption and oxidation of sugar, since the basal

part of a branch of which the tip alone is fumigated retains its starch. These authors also found that the catalases of plants are markedly inhibited by cyanide. Kastle and Loevenhart (11) worked with plant oxidases which turn tincture of guaiacum blue, and found that such oxidases are inhibited by cyanide. Schönbein and Schär observed (17) that the power of plant tissues to reduce nitrates to nitrites is greatly interfered with by cyanides. Schafer (20) extracted from the bodies of insects oxidases which turn guaiacum blue and give the indophenol reaction, reductases which reduce methylene blue, and catalases. Hydrocyanic acid was found to retard the activity of all three classes of enzymes reversibly. Of particular interest are the observations of Raubitschek (21) and Schultze (22) on the indophenol reaction. When a mixture of dimethyl-*p*-phenylenediamine and α-naphthol is applied to cells or tissues a deep blue precipitate of indophenol results in certain regions. As the reaction is an oxidation it occurs only where oxidations are taking place and has therefore been used to demonstrate the location of oxidases in cells. The authors mentioned have demonstrated that the indophenol reaction does not occur when cyanide is present. Sections made by the freezing method of organs of animals killed by narcotics, asphyxia, carbon monoxide gas and various other means all show the indophenol reaction but sections of organs from animals killed by potassium cyanide (placed in the mouth) give no trace of the reaction. The result is due to the inhibition of oxidases by the cyanide.

The cyanides therefore have a markedly depressing and inhibiting effect upon those groups of enzymes which are generally believed to bring about the oxidation processes in organisms.

As is to be expected, the cyanides have less or no effect upon that class of enzymes whose mode of action is hydrolytic. The observations of various investigators do not, however, agree very well. According to Fiechter (12), even very concentrated solutions of HNC have no effect upon the digestion of fibrin by pepsin, although a 1:2500 solution markedly retards the digestion of egg white by pepsin; a 1:200 solution was required to produce any retardation of the activity of salivary and pancreatic diastase. Fiechter was of the opinion that in concentrations of this strength the acidity was probably the chief factor. Butkewitsch (23) reported that trypsin from the pancreas is very resistant to the action of HNC and that certain proteolytic enzymes obtainable from the seeds of many plants are not only not inhibited by cyanide, but are accelerated. He thought that the first stages of proteolytic splitting were accelerated while the later stages were retarded, but

also suggested that the result might be due to the acidity of the hydrocyanic acid. Similarly Vines (24) found that 1 per cent HNC has no effect upon the activity of the proteolytic enzyme found in the pitchers of the pitcher plant, although the activity of glycerin extracts of the walls of the pitcher was slightly retarded by cyanide. The enzyme papain, a protease from the papaw, is remarkably accelerated by HNC, according to Mendel and Blood (25) and Frankel (26), the effect being due to the NC radical and not to the hydrogen ion. In contrast to these results are those of several investigators who report an inhibiting effect of cyanide upon similar enzymes. Abderhalden, Cammerer and Pincussohn (27), working with an enzyme from yeast which splits polypeptids, found that its activity was accelerated by very low concentrations of KNC (about 1/30,000 to 1/150,000 per cent), first accelerated and then retarded by less dilute solutions, and greatly retarded by stronger solutions. Chittenden and Allen (28) found that KNC prevents the digestion of fibrin by pepsin (a result exactly contrary to that of Fiechter), and retards the action of trypsin and ptyalin. Mercuric cyanide had similar effects. Some enzymes, however, were found by them to be accelerated by cyanides, or first accelerated and then depressed. Hahn and Geret (29) reported that HNC retards the activity of a trypsin-like enzyme from yeast. These conflicting results are, I think, reconcilable on the basis of the same suggestion which I have made with reference to the relation of cyanides to chemical reactions,—that whether the reaction shall be accelerated or retarded depends upon the nature of the compound formed with cyanides and hence upon the nature of the reacting material, in this case, the enzymes. The accelerating effect of dilute solutions of cyanide and the retarding effect of stronger solutions on some enzymes is suggestive since it resembles the effect of cyanides, narcotics and many toxic substances on intact organisms, and has also been observed in purely chemical reactions.

Numerous instances are at hand of the depressing and retarding effects of the cyanides upon physiological processes in general; in most cases, these effects can be most reasonably referred to depression of oxidation. The symptoms of cyanide poisoning in vertebrates markedly resemble those of asphyxia. Geppert (6) has described in detail the symptoms in mammals; the respiration is first greatly increased in rate and depth; defecation, vomiting and loss of muscular control follow; breathing becomes more difficult but is still rapid; violent convulsions occur; finally the convulsions cease, the respiration slows down and

death occurs with a final increase in respiratory rate. Dontas (30) reports similar symptoms in frogs poisoned with cyanide: irregularity of respiration followed by a marked decrease in rate; tremblings of the muscles; cessation of breathing and disappearance of reflexes. According to Drzewina (31) fish exhibit all of the symptoms of asphyxia when placed in cyanide solutions; they swim violently, come to the surface for air and finally die with decreased respiration. In vertebrates then the symptoms chiefly concern the central nervous system, a fact of particular significance in view of the high metabolic rate of the nervous system which renders it peculiarly susceptible to asyphyxiating substances. Particular emphasis has been placed upon the depressing effect of cyanide upon the respiratory center but this is misleading, as all portions of the brain are affected, as evidenced by vomiting (vomiting center), loss of muscular control (cerebellum?), loss of consciousness (cerebral hemispheres), disappearance of the corneal reflex (sensory nucleus V, motor nucleus VII), etc. Grove and Loevenhart (32) and Gasser and Loevenhart (33) have studied the effect of cyanide upon the medullary centers of mammals. They found that small doses of cyanide stimulate these centers, larger doses first stimulate and then depress. The respiratory center is most sensitive, vasoconstrictor center next and cardio-inhibitory center least sensitive to cyanide. Adding carbon monoxide, hydrogen or nitrogen to the inspired air, clamping the carotid arteries, stopping the artificial respiration or any method which reduces the oxygen supply to the brain produces effects identical with those due to injecting cyanide; that is, cyanide acts in the same manner as lack of oxygen. Loeb in many of his studies has proved that the effect of cyanide on sea-urchin eggs is identical with lack of oxygen: for example, cytolysis of sea-urchin eggs after exposure to hypertonic solutions is prevented by potassium cyanide or an atmosphere of hydrogen (34); fertilized eggs will not develop either in an atmosphere of hydrogen or in the presence of KNC (35), a 1/2000 per cent solution completely but reversibly preventing development, while weaker solutions only retard it; cyanide prevents the parthenogenetic action of hypertonic sea water, for which oxygen is likewise necessary (35); unfertilized eggs are much less susceptible to cyanide than fertilized and developing ones (35), and as is well known consume much less oxygen than the latter; the toxic effects of a number of solutions upon sea-urchin eggs can be prevented either by withdrawing oxygen from the toxic solution or adding cyanide to them (36), (37). The circulation of the protoplasm in plant cells, for which oxygen is nec-

essary, is reversibly stopped by hydrocyanic acid (38). The body temperature of mammals, which is due to oxidation, falls reversibly in non-lethal cyanide poisoning (39). Amberg, Loevenhart and McClure (40) have observed that certain experimentally induced inflammations are greatly benefited or prevented by the application of substances which yield oxygen readily, and cyanide aggravates the inflammation and counteracts the curative effects of these substances. Dontas (30) has made an interesting study of the action of sodium cyanide upon the nerves and muscles of the frog. He found the central nervous system to be most susceptible, the peripheral nerves less susceptible and the muscles the least susceptible of the neuromuscular apparatus. The conductivity of nerves was found by him to be completely but reversibly abolished in the proper concentrations of cyanide. The current of action and the current of injury completely disappear in nerves immersed in cyanide of proper strength, and return to their former value when the cyanide is washed out. I have discussed elsewhere the relation of these currents to metabolic rate (41). Strong sunlight is injurious or even fatal to many of the lower invertebrates, presumably because it accelerates metabolic processes too greatly; Drzewina (31) has found that these effects of light are abolished in the presence of cyanide. Moore and Willaman (19) state that photosynthesis is decreased in plants fumigated with HNC gas and, as is well known to plant physiologists, is similarly affected by lack of oxygen.[2] They further found that cyanide interferes with the consumption of sugar in plants, presumably an oxidation. According to Skanischewsky (42), cyanides decrease the power of animals to perform simple oxidation such as the formation of phenol from benzene. Budgett (43) has noted that lack of oxygen and potassium cyanide (also other poisons) produce identical structural and death changes in Protozoa; and he figures a disintegration gradient in *Oxytricha* in lack of oxygen and cyanide which is identical with the gradients which we have described for many of the lower organisms killed in cyanide. Planaria also exhibits the same death gradient in lack of oxygen as it does in cyanide. Cyanide prevents the germination of seeds and fungus spores, and completely inhibits their growth (13), (17), (44); dilute solutions may accelerate these processes. For growth and germination of such materials, oxygen is of course indispensable.

[2] Statements of this kind are made on the authority of Jost's *Plant Physiology*.

These instances, including a variety of physiological processes, all strongly suggest that there is a direct relation between cyanide and metabolic rate. The action of cyanide upon these processes is similar and often identical with the effect of absence of oxygen; and is most striking upon processes and conditions known to require much oxygen.

It must always be borne in mind that the concentration of cyanide employed in such experiments is of the greatest importance, since dilute solutions often produce the opposite effect from stronger solutions.

The final group of data which I wish to present includes those instances in which direct measurements of the respiratory exchange in the presence of cyanide have been made. This kind of evidence is of course the most convincing and I have therefore made an effort to collect all of the cases of this kind extant in the literature. The impetus to experimentation upon this subject was given by the discovery of Claude Bernard (45) that the venous blood is red in cyanide poisoning. This was confirmed by Gaethgens (46) who notes that in cyanide poisoning in rabbits the blood in the small vessels of the ears and mucous membrane of the lips and in the jugular vein is bright red, and that the venous side of the heart (as viewed through an opening made in the diaphragm) is of the same bright red color as the arterial half. The observation has of course been made repeatedly since.[3] Claude Bernard did not explain the red color; but with his usual perspicuity he determined that the action of cyanide upon the blood is not the same as that of carbon monoxide, since blood when mixed with cyanide will not turn red unless air is present. In other words, in all probability, the red color is simply due to oxyhemoglobin. Hoppe-Seyler, Preyer and their students were much interested in this question of the causation of the red color of the venous blood in cyanide poisoning, and they believed it was due in part at least to the formation of a compound between cyanogen and hemoglobin. But Hoppe-Seyler (48) was unable to prove that any such compound exists in the circulating blood when cyanide is present, since such blood gives the same spectrum as

[3] Chio (47) presents a theory that the red color of the venous blood in cyanide poisoning is due to the action of cyanide on the walls of the veins so that the venous blood flows so fast through the veins that the cells do not have time to withdraw oxygen from it! The idea seems absurd, as there is no evidence of vasomotor fibers in the walls of the smaller veins. Chio's further contention that cyanide acts by disturbing the neutrality mechanism is pertinent only to the higher animals, whereas cyanide produces similar effects on all animals.

normal blood and yields normal hemoglobin crystals. This question was settled by the experiments of Zeynek (49) who proved that hemoglobin will not unite with cyanide, and oxyhemoglobin unites with it only after heating several hours at body temperature; and it is now universally recognized that the action of cyanide on vertebrates is not due to such union.

The earlier experiments on the rate of oxygen consumption in cyanide poisoning were inconclusive because of inadequate technique. The first reliable experiments were those of Geppert (6), who pointed out the inadequacies of the earlier methods and used improved methods. Geppert found that the oxygen consumption of mammals is increased during the first stages of cyanide poisoning and later falls markedly. When all of the symptoms of poisoning have developed, the oxygen consumption is always less than normal, in spite of the fact that the animal is in violent muscular convulsions and that the respirations are greatly increased in rate and depth. It is quite impossible therefore to ascribe the decrease in oxygen consumption to effects upon the respiratory center, for the decrease appears while the respirations are enormously accelerated and the amount of air passing through the lungs is much greater than under normal conditions. It was further conclusively proved by Geppert that the red color of the venous blood is due to oxyhemoglobin. Such red venous blood was found by him on analysis to contain as much or nearly as much oxygen as the arterial blood, and the oxygen content of the arterial blood was the same as usual. The arterial blood therefore takes up oxygen in the lungs in the usual amount but the tissues do not withdraw this oxygen. Geppert further demonstrated that the oxygen in the venous blood in cyanide poisoning is not held or bound in any other than the normal way, since the oxygen can easily be withdrawn from the blood, as by allowing the animal to breathe hydrogen. After recovery of the animals from non-lethal doses of cyanide, the oxygen consumption and the color of the venous blood return to normal. I believe that anyone who will take the trouble to read Geppert's paper carefully will be convinced that he has avoided every source of error and answered all objections, and that there is no escape from his conclusion that in the animals with which he worked the oxygen consumption of the cells is directly decreased in the presence of cyanide.

A considerable number of similar experiments have been performed since on various living materials which entirely support Geppert's

conclusion. Schroeder (50) measured the effect of KNC upon the rate of oxygen consumption and carbon dioxide output in *Aspergillus*. He found that the oxygen consumption is reduced 50 to 94 per cent according to the concentrations used, and the carbon dioxide production also greatly diminished. Recovery and return to the original rate of respiratory exchange occurred from all except the strongest concentrations. Skanischewesky (42) determined the action of a number of nitrils on mammals and found that all of them decreased both the rate of oxygen consumption and of carbon dioxide production. Complete recovery occurred. Since the effect of the various nitrils was the same, the author concluded that the NC group was responsible for the result. Vernon (51), working on isolated kidneys perfused with Ringer's solution, found that the oxygen consumption of such preparations is greatly decreased when HNC is added to the Ringer's solution and gradually rises again when the cyanide is washed out. Batelli and Stern (52) studied the respiratory exchange of minced organs and tissues of vertebrates, and found that such preparations consume oxygen and give off carbon dioxide in large quantities for several hours after death, the respiratory exchange of course gradually diminishing. They determined the action of potassium cyanide on beef liver and muscle, horse muscle and brain and pigeon muscle, performing sixteen experiments in each of which one to five concentrations of cyanide ranging from 1/12 to 1/10,000 mol. were employed. In all cyanide-containing solutions, a decrease in both oxygen consumption and carbon dioxide production was observed, the amount of decrease depending upon concentration. Whether recovery occurs was not stated by the authors. Warburg (53) found that the oxygen consumption of sea-urchin eggs and red blood corpuscles of geese is decreased 50 to 70 per cent in the presence of potassium cyanide. Warburg (54), Fiechter (12) and others noted that the oxygen consumption of yeast is decreased by cyanide and nitrils. Loeb and Wasteneys (55) also demonstrated that cyanide decreases the rate of oxygen consumption in sea-urchin eggs. In sponges,[4] I showed that dilute solutions of KNC increase the rate of oxygen consumption, slightly stronger ones first increase it and then decrease it, and stronger solutions decrease the rate to a marked degree (1). In all of my experi-

[4] I take this opportunity of correcting an erroneous statement made in that paper regarding the taxonomic position of Suberites, the sponge employed. It is not a calcareous sponge, as stated, but a siliceous sponge, belonging to the Monaxonida.

ments complete recovery and return to original rate of oxygen consumption was observed. Onaka (56) measured the oxygen consumption of red blood corpuscles of geese in cyanide. In 1/1000 mol. solution no oxygen was consumed; in 1/10,000 mol., the oxygen consumption was about 20 per cent of the normal; and in 1/20,000, about 40 or 50 per cent of the normal. The result was reversible, recovery occurring in about one and a half hours. Onaka further noted that cyanide does not affect the oxygen-carrying function of the red blood corpuscles, since corpuscles in cyanide will lose their red color when hydrogen is bubbled through, and regain it when oxygen is again admitted. Shafer (57) found that hydrocyanic acid gas (and other volatile insecticides) decreases the rate of oxygen consumption and carbon dioxide output in *Passalus cornutus*, a common Lucanid beetle.

These experiments, covering a wide range of materials and performed independently by a number of investigators, furnish a sound basis for the conclusion which has been generally drawn from them that cyanides directly decrease the rate of oxygen consumption of protoplasm. The chief objection raised against this conclusion is the one advanced by Carlson (58) and others,—that anaerobic organisms and tissues are just as susceptible to cyanide as aerobic ones. To this objection there is, I think, an adequate answer. The energy-producing processes of anaerobic organisms are not in reality different from those of aerobic ones. Whereas the latter employ extramolecular oxidations, the former make use of intramolecular ones, such as the reduction of sulphates, nitrates, etc. A case in which the evidence is complete is that of yeast. Yeast can consume oxygen in the ordinary way, deriving energy from ordinary protoplasmic respiration; it can also carry out an intramolecular oxidation, the splitting of sugars into alcohol and carbon dioxide. The latter process is in fact accelerated by lack of oxygen, showing that it can take the place of ordinary respiration. Cyanides by virtue of their reducing powers ought to interfere with both types of oxidations; this is indeed the case, as was long ago pointed out by Fiechter (12). In fact the intramolecular oxidation is much more sensitive to cyanide than the extramolecular one, perhaps because the former is a simpler reaction. It is worthy of note in this connection that cyanides also interfere with the oxidations of sugar in vitro, as by Fehling's and similar solutions.

Since cyanides decrease the rate of oxygen consumption, it follows that cells or organisms or parts of organisms which have the highest rate of respiratory exchange will be more susceptible to cyanide and

will therefore die in lethal concentrations of cyanide faster than parts or organisms respiring less actively. On this basis the death gradients in organisms and the differences in time of death of different individuals or parts of organisms killed in cyanide have been interpreted by workers in this laboratory (3) as due to differences in metabolic rate, or more specifically, rate of oxygen consumption. This interpretation, therefore, was neither invented, devised nor assumed by these workers, as certain critics of it persist in saying, but was simply the logical conclusion to be drawn from the large mass of experimental evidence which I have summarized here. In fact, potassium cyanide was chosen by Child (59) for the purpose of demonstrating such gradients after he had become convinced from other evidence that they existed because cyanide was generally accepted by physiologists and pharmacologists as a depressor of metabolic processes. We have however tried wherever the nature of the material permitted to check the susceptibility data by direct measurements of carbon dioxide production (determinations of oxygen consumption will be made next). The carbon dioxide production has been determined by the biometer and by Haas's indicator method (60). In this way Child has proved that individuals or parts of Planaria which are more susceptible to cyanide also invariably give off more carbon dioxide than less susceptible ones.[5] All conditions which are known to increase oyxgen consumption and carbon dioxide output in organisms, such as motor

[5] A special statement is required with regard to the conditions in starvation and feeding. Starvation gradually increases the susceptibility to cyanide. However, the total metabolism, as determined by CO_2 production, decreases during the early stages of starvation and increases only in the later stages. This apparent discrepancy between susceptibility and metabolic rate is quite simply explained. Susceptibility is commonly measured by the time of death of the surface of the body, little attention being paid to the digestive tract. Total metabolism, however, involves also the metabolism of the digestive tract which, as is well known, is greatly increased by feeding; this increase is quite noticeable in Planaria also. Therefore, in the first stages of starvation, as the activity of the digestive tract is diminished, the total metabolism falls, whereas the susceptibility to cyanide (of the body surface) remains the same or increases. In the later stages of starvation, both susceptibility and total metabolism increase. The increased activity of the digestive tract through feeding can be detected by cyanide also, provided one's attention is directed to this particular point, that is, the digestive tract often disintegrates in fed animals before the body wall does. The term susceptibility, however, as used by us, generally refers to the susceptibility of the surface only. The full report of Child's work on starvation and feeding is now in press in this journal.

activity, injury, growth and regeneration, rise of temperature, also increase the susceptibility to cyanide. Young and growing regions of plants and animals, or young organisms carry on metabolism more actively than older ones and are likewise more susceptible to cyanide. The same facts are known for developing eggs, the oxygen consumption and heat production and also susceptibility to cyanide increasing from fertilization up through the gastrula stage (61). Alvarez (62) has found the same conditions to obtain in the digestive tract. The upper portions of the intestine have a greater inherent irritability, a faster rate of contractile rhythm, contain more catalase, give off more carbon dioxide and are more susceptible to cyanide than lower regions. Allee (63) has determined similar facts with regard to isopods. In these organisms, a number of conditions which are known to accelerate metabolic rate were shown to increase the positive rheotactic response, the susceptibility to cyanide, and the carbon dioxide production (64).

The idea has been advanced by some workers, recently again by B. L. Lund (65), that differential susceptibility to cyanide is due to differential permeability. There has never been the slightest doubt in the minds of either Professor Child or myself that a relation exists between permeability and susceptibility to cyanide; we have certain ideas as to the nature of this relation but have not yet subjected them to experimental proof. The explanation given by B. L. Lund, however, that differential susceptibility is due merely to an easier penetration of cyanide into more permeable regions, is altogether too simple and quite untenable in the face of the following facts. *a*, The same susceptibility gradients appear in organisms killed with neutral red or other colored substances as in cyanide. In these cases the colored substance can be observed entering the cells. All cells or parts in many cases stain equally red, proving that the dye has penetrated throughout; nevertheless death occurs as a gradient identical with the gradient in cyanide. *b*, Death gradients like those in cyanide occur in organisms killed by lack of oxygen or extreme temperatures. *c*, The death gradient in dilute solutions of cyanide or other reagents is exactly the reverse of that obtained in strong concentrations. *d*, The intestine of a recently fed Planaria is often more susceptible to cyanide than the body wall; in other words, the cyanide *passes through the body wall, leaving it intact*, and attacks the intestine. *e*, Acids are generally believed to decrease permeability; nevertheless, non-lethal concentrations of acids, unless very dilute, increase the susceptibility to cyanide.

In view of the fact that Lund's experiments are the only ones on record in which quantitative measurements have failed to show a

decrease in the rate of oxygen consumption in the presence of cyanide, it is necessary to consider his material and methods. Both are objectionable in several respects. In the first place, Paramecium is very resistant to cyanide; in fact, it is the most resistant animal, except Amoeba, which we have encountered. It is therefore not to be expected that cyanide in concentrations ordinarily employed would greatly affect the rate of oxygen consumption. It is therefore necessary to use high concentrations of cyanide to produce any effect, and this Lund has not done, since by his method the animals must be exposed to the cyanide for long periods of time, with the result that relatively dilute solutions have to be employed. In his table 3, where the strongest solutions are employed, he happened to use a particularly resistant lot of Paramecia. It seems quite probable that most of the concentrations used by Lund were too dilute to produce a measurable effect. This is further indicated by the fact that in three of the four columns of determinations given, the oxygen consumption of Paramecium was accelerated in the strongest solutions used. Such acceleration occurs only in solutions relatively dilute for the organism concerned, and it indicates that these strongest solutions are to be regarded as dilute for Paramecium, and the others as too dilute to produce any effect. It is further possible that the alkalinity of the cyanide may have a stimulating effect. The oxygen consumption of Protozoa is so slight that by Lund's method it is necessary to inclose the organisms for many hours in small bottles. While this procedure might be satisfactory under normal conditions, it is certainly objectionable where a drug is present because the initial effect of the drug might be quite the opposite of the final effect, and the oxygen determination at the end of a number of hours (10 to 51 in Lund's experiments) is really meaningless, since it is the average of all of the changes which have ensued in the bottle during the experiment. Lund analyzes the bottles with the Paramecia still inclosed in them, so that the error due to the iodine absorption by the dead animals is relatively large. Thus in his table 2, the error due to iodine absorption is actually one-fourth to one-third of the apparent oxygen consumption. Furthermore, the iodine absorption is measured only at the beginning of the experiment; Lund does not know what it is at the end of the experiment, that is, the control and the experimental bottles are not really comparable. Lund says that Paramecia do not alter the iodine absorption of water but as he gives no reasons in support of this statement, it must be regarded as pure assumption. I do not think it is really possible to determine

the iodine absorption in the presence of cyanide in bottles of different oxygen content, and hence this source of error which is considerable in Lund's experiments cannot be controlled by his method. One would further like to know what effect upon the oxygen consumption, the production of CO_2 and other metabolic products may have in organisms respiring for hours in small closed bottles; and how the increasing acidity of the water may alter the equilibrium conditions with cyanide. There is no doubt that decreasing the alkalinity also decreases the susceptibility to cyanide. It is also possible that acclimation to cyanide is involved in the longer periods of exposure. One notices that the animals respire relatively less in the longer periods than in the shorter ones; although this fact is neither mentioned nor explained by Lund, it is presumably due to starvation, although it might also be due to depression from the accumulation of metabolic products in the water.[6] In fact there are so many sources of difficulty involved in the method used that the results cannot be interpreted with certainty. A final great lack in Lund's experiments is that no figures are given anywhere of the normal rate of oxygen consumption of the same lots of Paramecia. In reference to one table, Lund says that the average oxygen consumption was found to be the same in tap water as in cyanide but more definite data upon this point are desirable. It seems to me that it is really essential in such experiments to determine at least once, preferably two or three separate times, the normal rate of oxygen consumption of a particular lot of animals, and then determine the oxygen consumption of the same or a comparable lot of animals in cyanide. It is further desirable that determinations should be made with higher concentrations of cyanide and shorter periods of exposure.

I do not think one can accept Lund's conclusion in regard to table 4, that the cessation of oxygen consumption in cytolysing solutions of KNC is due entirely to the cytolysis and not to the cyanide. We have plenty of evidence that cytolysed and disrupted cells not only use up oxygen but may even use up more oxygen than intact cells (owing to the stimulation of injury). Some of this evidence may be found in Vernon's *Intracellular Enzymes*, pp. 138ff. Thus Batelli and Stern (52) and Harden and MacLean (67) found that a variety of minced organs and tissues of vertebrates use up oxygen and give off carbon dioxide. Warburg's experiments are of great interest. He found (68) that when the plasma membrane of red blood corpuscles is

[6] Woodruff (66) has shown that the metabolic products excreted by Paramecium have a depressing effect upon it.

destroyed by freezing and thawing or by distilled water, the cell remnants consume oxygen and give off carbon dioxide at the same rate as intact cells or often more rapidly. Liver extracts containing no intact cells also consume oxygen and give off carbon dioxide (69). Unfertilized sea-urchin eggs rubbed up with sand or broken by shaking or cytolysed by diluted sea water still use up nearly as much oxygen as intact eggs, and by the most favorable methods of preparation may use even more (70). Suspensions of granules obtained from unfertilized eggs respire faster than a corresponding quantity of intact unfertilized eggs. In support of his contention, Lund has quoted only one reference, that to Warburg's work on *fertilized* eggs. If Lund had read Warburg's original paper (70) instead of the general paper to which he refers, he would have found that he has misunderstood Warburg's statements. Warburg's results with fertilized eggs were really these. Fertilized eggs respire several times faster than unfertilized eggs. This additional oxygen consumption due to fertilization is lost when fertilized eggs are broken up by the above methods. Nevertheless such disrupted fertilized eggs respire as much as or a little more than an equal quantity of cytolysed *unfertilized* eggs. In other words, it is only the stimulation due to fertilization which is dependent upon structure and is lost upon cytolysis, the basic metabolism continuing just the same. Lund is therefore left without any support for his contention that oxygen consumption ceases upon cytolysis, except his own experiments with high oxygen pressure. It is, however, manifestly unfair to use oxygen as a cytolysing agent in order to prove that oxygen consumption ceases upon cytolysis. In the absence of further facts with regard to the effect of cytolysis upon oxygen consumption in Paramecium and in consideration of the facts quoted above with regard to other material, one may conclude that the failure of Paramecium to respire after cytolysis by cyanide is due in part at least to the action of the cyanide, and constitutes an indirect proof that cyanide decreases the rate of oxygen consumption in Paramecium. It is manifestly ridiculous for anyone to maintain that fragments of protozoan cells do not respire, since such fragments swim about for a long time and retain contractile power.

METHOD

The present experiments were performed upon *Planaria dorotocephala*, a turbellarian flatworm common in certain spring-fed pools near Chicago. Large numbers of these animals are kept on hand in

the laboratory in covered dish pans. They are fed three times a week on liver. As the city water is chlorinated and has been found injurious to organisms, we use only well water for all stock cultures and experiments. This water is obtained from a well sunk in the basement of the zoölogy building. It is practically oxygen-free, except when the pump sucks a little air in, and oxygen is therefore added to it by running it through a simple aerating device. This brings the oxygen content up to about 3 cc. per liter. For my experiments I have still further aerated it by shaking it in a large bottle, obtaining by this means an oxygen content of 5 cc. or more per liter.

The apparatus used was simple and similar to that employed in my previous experiments along this line. A 1000 cc. Erlenmeyer flask was provided with a tightly fitting rubber stopper perforated with three holes. Through one of these holes was inserted a glass tube reaching to the bottom of the flask through which water was siphoned in; through another a similar tube also reaching to the bottom through which water was siphoned out; and the third hole was used to admit air during the siphoning, being closed at other times by a short glass rod. Screw clamps on rubber tubing inserts were provided at necessary places. The exit tube was bent over and extended several inches below the bottom of the flask. This apparatus can be made air-tight without the slightest difficulty, as proved by the facts that no water would siphon out of it when all other exits were closed as in the experiments, and if it was filled full of warm water, all exits closed, and allowed to cool, the flask would break as soon as the temperature had fallen two or three degrees, owing to the contraction of the water.

For each experiment two or three hundred worms were used. These were selected at random from the general stock and their heads cut off at least several hours before they were to be used. Such decapitated worms remain absolutely quiet, if they are shaded from direct light, throughout the course of an experiment. There is therefore no possibility that differences in motor activity are involved in the results. No difference can be observed in the behavior of these worms in normal and cyanide-containing water.[7] Each lot of worms prepared in this

[7] Animals which have been exposed to high concentrations, as 1/2000, show on stimulation a decreased ability to respond, and are not as successful in clinging to the substratum as the normal worm. These effects disappear shortly on return to water, and are not observable in concentrations such as 1/25000 which lower the oxygen consumption to a considerable degree. The action of cyanide on the irritability of organisms is a separate problem, which I shall shortly undertake.

manner was used in several different experiments, being allowed to recover in water between successive experiments. It was thus known that the animals recovered completely and regained their former rate of oxygen consumption after exposure to cyanide.

The procedure in each experiment was as follows: Several liters of shaken well water were poured into a large elevated receptacle. The worms were put into the Erlenmeyer flask. To prevent them from being carried up the exit tube a piece of well-washed cheesecloth was tied over its end. Water was siphoned from the receptacle into the flask, allowing the latter to overflow for some time so that the worms, the flask and all of the tubes were thoroughly washed. At the same time a sample of the same water was collected from the receptacle in the same manner into a 250 cc. narrow-mouthed bottle with ground glass stopper to serve as control. All openings to the flask were then closed and it and the control bottle were placed in a large pan of water kept constant to 0.5 of a degree, and regulated to the same temperature as the water in the flask. After one hour the flask was taken out and shaken thoroughly to insure an even distribution of its oxygen content. Water was then siphoned from it into a 250 cc. narrow-mouthed bottle, allowing this to run over for some time so as to obtain a sample which had not come in contact with air. This sample and the control sample were than analyzed for oxygen content, the difference between them being, of course, the amount of oxygen consumed by the worms.

This procedure was repeated twice with normal water, so that three separate determinations of the normal rate of oxygen consumption of the same lot of worms were obtained. The desired amount of cyanide was then added to the water in the receptacle (which was of course refilled for each hour determination) and three more separate successive hour measurements were made of the rate of oxygen consumption of the same worms in the presence of cyanide.

The potassium cyanide was weighed out fresh for each hour determination. In the case of the very dilute solutions, where the amount of cyanide to be weighed was so small that absorption of water during the weighing might introduce a considerable error, stronger solutions were made (fresh each time), and the necessary amount of these stronger solutions was poured into the receptacle. As both control and experimental water comes from the same receptacle, they are identical in composition, the control always containing cyanide and the same amount of cyanide when the experimental water contains it. It is quite essential that the control water contain the same amount of

cyanide as the experimental water, since cyanide affects the method of oxygen analysis.

It might be supposed that by this method oxygen might enter the water during the taking of the sample, since it is necessary to admit atmospheric air into the flasks during the siphoning. I have found by test, however, that this is not the case and that there is not the slightest error due to this circumstance. I have made triplicate analyses of the same water, two of the three samples being collected by passage through the same Erlenmeyer flasks used in the experiments, and the third sample taken directly into the 250 cc. bottle. The water used in these tests always contained less oxygen, often very much less, than the water encountered in the experiments, including that in which worms had been respiring. The analyses of such triplicate samples showed that they contained the same quantities of oxygen, within 0.01 to 0.04 cc. of oxygen per liter, except in the water with exceedingly low oxygen content where the variation in the three samples ranges to 0.09 cc. per liter. But even this variation cannot be regarded as due to the process of siphoning but rather to the general manipulation, since in each of the two tests conducted with very low oxygen water, one of the siphoned samples is as close to the direct sample as in the cases where water of higher oxygen content was used. It may therefore be safely said that no oxygen is absorbed by water having an oxygen content below that corresponding to a state of equilibrium with the air by siphoning it with admission of air. The data of these tests are given in the accompanying table 1, which includes all of the tests made. The method may therefore be regarded as perfectly adequate for the purpose. In the early part of the work I used an Erlenmeyer flask for control and collected the control sample from it in the same manner as the experimental sample was collected, but as these tests showed that such a procedure is unnecessary, the control sample was thereafter collected directly into the bottle in which the analysis was carried out.

The samples of water collected in this manner were analyzed by Winkler's method, following the procedure given by Birge and Juday (71). The 1/100 normal solutions recommended by them certainly permit of greater accuracy than the 1/40 normal solutions commonly in use. I have also found the suggestions of McClendon (72) helpful. The thiosulphate was kept in a large darkened bottle, provided with a soda-lime tube to keep out carbon dioxide and fed by siphon into a burette provided with a side arm. It was standardized at intervals

with permanganate as described in my first paper (1). As a matter of fact, however, the actual normality of the thiosulphate solution is of little consequence provided it does not change appreciably during the titration of all of the samples from one experiment.[8] In most of the experiments, all of the samples were analyzed within a few hours of each other, but in some, owing to the shortness of the days, some of the samples had to be left until the next day to be titrated. As Birge and Juday noted, such delay introduces no error into the titration.

The Winkler method depends upon the setting free of iodine. If anything is present in the water which takes up iodine, an error will consequently be introduced into the results. Do Planarians add any

TABLE 1

Tests of the accuracy of the apparatus; analyses in triplicate of the same water, two samples having been passed through the apparatus as in the experiments, and the third sample taken directly

KIND OF WATER	SAMPLE THROUGH APPARATUS	SAMPLE THROUGH APPARATUS	DIRECT SAMPLE
	Oxygen content, cubic centimeters per liter		
Slightly aerated well water	1.28	1.30	1.30
Slightly aerated well water	1.93	1.94	1.90
Standing well water	4.09	4.07	4.09
Standing well water	3.95	3.98	3.97
Standing well water, stirred	4.54	4.55	4.53
Unaerated well water	0.57	0.63	0.54
Unaerated well water	0.51	0.59	0.60

iodine-absorbing material to the water? This can be determined quite simply as follows: Two hundred and fifty cubic centimeters of the water to be tested are acidified with about ½ cc. of concentrated hydrochloric acid, a known amount of N/100 iodine run into it from a burette, and the water then titrated with thiosulphate. The difference between the amount of iodine added and the amount recovered represents the amount of iodine absorbed. In this way I have found that the well water contains some iodine-absorbing material as compared with distilled water blanks, but that well water which has contained worms for one hour has less iodine-absorbing power than the ordinary well

[8] Thiosulphate solutions to which NaOH has been added as suggested by Birge and Juday show practically no deterioration.

water. That is to say, the oxygen consumption of the worms is actually a little greater than appears from the analysis since in the experimental bottles less iodine is absorbed than in the control bottles. I have not made this correction, since it was somewhat variable, and I thought it not worth while to undertake the great amount of extra labor which would have been required to determine its amount for every sample. The amount of this correction was not large enough to be of any consequence for the general conclusion to be drawn from the experiments.

As I have already pointed out, cyanide has marked iodine-absorbing power. If the above procedure is repeated with water containing cyanide, it will be noticed at once that as the iodine is run into the solution the color fades out to some extent. If such solutions are titrated with thiosulphate, it will be found that it is almost impossible to come to an end point, indicating that a condition of changing equilibrium exists between the various substances present in the solution. Since there is no difficulty in determining the end point by Winkler's method in solutions containing cyanide, it is evident that during the time occupied in performing Winkler's analysis, a condition of static equilibrium is reached. I have not been able to devise any way of finding out what the iodine absorption in cyanide-containing water really is. Since, however, in my experiments, the control water always contains cyanide when the experimental water does, and this is an absolutely essential point, the error due to absorption of iodine by cyanide may be regarded as eliminated. There remains still the possibility that the effect of the worms on the iodine-absorbing power of the water may be different in cyanide than in normal water. Such evidence as I have been able to obtain upon this point indicates that in cyanide also the worms reduce the iodine absorption of the well water. It is therefore likely that all of the figures of the oxygen consumption of the worms are a little too low. The results are, however, so very consistent, definite and striking that it cannot be supposed that these small sources of error could in the least alter the conclusion to which they lead.

EXPERIMENTAL RESULTS

The results of my experiments are recorded in the accompanying tables. These experiments have not been selected for presentation but they represent all that I performed with two or three exceptions where

accidental difficulties arose. In every experiment which I carried out, the result was perfectly clear-cut. There were no exceptions. In all the oxygen consumption was decreased, the amount depending upon the concentration of the cyanide. As already stated, each experiment, except in one or two cases, consists of six one-hour measurements of the oxygen consumption, the first three in normal water, the second three in cyanide-containing water, all six being performed on the same worms which remain in the flask throughout the experiment. To save time, two lots of worms have generally been run with one control. The six figures of the oxygen consumption of a lot of worms are given in the vertical columns in the tables. Each figure represents, of course, the difference between the oxygen content of the control bottle and the oxygen content of the sample drawn from the experimental bottle. Several times samples were lost (and if this happened to be the control bottle, two determinations were lost thereby), through accidents of one kind and another; such loss is indicated by a blank in the table. In connection with the most dilute solutions, I measured the oxygen consumption during the first half-hour and second half-hour in cyanide instead of during the first hour, in order to discover whether or not there is any initial stimulating action of the cyanide. In connection with each concentration, some figures of the respiration after recovery are given. Although it was known that recovery always occurred since the same lot of worms was used several times, in fact until the animals had regenerated sufficiently to cause them to move about, the exact figures were not always usable because successive experiments were often performed at different temperatures, and because two lots of worms were always in use simultaneously and I did not always note from which of the two lots a particular set of figures was obtained. Recovery from the higher concentrations required more than 24 hours, with several washings, but occurred in less than 24 hours from the low concentrations. A slight decrease in oxygen consumption in a lot of worms kept several days without feeding will of course occur on account of starvation; for this reason the oxygen consumption after recovery must be expected to be slightly less than it was during the original experiment. This is quite evident when the oxygen consumption is determined more than 48 hours afterwards. In the first two or three experiments the temperature was not kept constant, accounting for a larger variation in successive determinations in those experiments, but with these exceptions, the temperature varied less than 0.5° throughout the course of the experiment.

Extended comment upon the data given in the tables is unnecessary. In all cases, the oxygen consumption is decreased in the presence of cyanide. This decrease ranges from 80 to 90 per cent of the normal

TABLE 2

Experiments with 1/1000 mol. KNC

NUMBER OF EXPERIMENT	5a	5b
TEMPERATURE	22°	22°
	O_2 consumed, cubic centimeters per hour	
First hour normal	0.34	0.32
Second hour normal	0.33	0.33
Third hour normal	0.38	0.37
First hour KNC*	0.04	0.08
Per cent decrease	89	77

* It is not possible to expose the worms to 1/1000 mol. solutions for more than one hour, as they disintegrate in two or three hours in solutions of this strength.

TABLE 3

Experiments with 1/2000 mol. KNC

NUMBER OF EXPERIMENT	1	2	13a	13b	18a	18b	25a	25b	27a	27b	33b
TEMPERATURE	Not constant	Not constant	22°	22°	23°	23°	23°	23°	21°	21°	21°
	Oxygen consumed, cubic centimeters per hour										
First hour normal	0.42	0.57	0.39	0.43	0.41	0.45	0.55	0.66	0.79	0.85	0.65
Second hour normal	0.38	0.70	0.47	0.48	0.48	0.49	0.61	0.71	0.87	0.94	0.62
Third hour normal	0.55	0.89	0.44	0.49	0.46	0.44	0.62	0.76	0.82	0.89	0.63
First hour KNC	0.11	0.14	0.07	0.08	0.07	0.08	0.12	0.12	0.14	0.16	
Second hour KNC	0.10	0.13	0.06	0.09	0.05	0.06	0.07	0.08	0.02	0.02	0.06
Third hour KNC	0.06	0.02	0.04	0.07	0.07	0.07	0.08	0.08	0.07	0.09	0.06
Per cent decrease	80	87	87	83	86	85	85	87	91	90	91
			After 48 hours						After 4 days		
Recovery*			0.43	0.45					0.72	0.79	

* Recovery figures are the average of three determinations.

in the highest concentrations to 5 to 15 per cent in the lowest. In general the dilute solutions are relatively more effective. Thus, there is little difference in the action of 1/1000, 1/2000 and 1/5000 mol.

solutions, while the 1/25,000 and 1/50,000 solutions are much more effective than would be expected. With the highest solutions, the

TABLE 4

Experiments with 1/5000 mol. KNC

NUMBER OF EXPERIMENT	3a	3b	4a	4b	12a	12b	24a	24b	26a	26b
TEMPERATURE	Not constant	Not constant	19°	19°	23°	23°	21°	21°	21°	21°
	Oxygen consumed, cubic centimeters per hour									
First hour normal	0.49	0.41	0.30	0.35	0.36	0.43	0.73	0.62	0.71	0.68
Second hour normal	0.43	0.34	0.33	0.34	0.38	0.37	0.86	0.76	0.88	0.91
Third hour normal	0.58	0.44	0.29	0.29	0.42	0.45	0.66	0.58	0.83	0.85
First hour KNC	0.17	0.11	0.06	0.06	0.16	0.08	0.19	0.19	0.14	0.24
Second hour KNC	0.21	0.17	0.03	0.03			0.13	0.09	0.13	0.15
Third hour KNC	0.10	0.08	0.09	0.09	0.05	0.06	0.11	0.10		
Per cent decrease	68	68	79	82	70	84	86	81	84	77
					After 48 hours		After 4 days		After 48 hours	
Recovery*					0.43	0.46	0.60	0.71	0.82	0.89

TABLE 5

Experiments with 1/10000 mol. KNC

NUMBER OF EXPERIMENT	6a	6b	7a	7b	11a	11b	29a	29b
TEMPERATURE	23°	23°	20°	20°	23°	23°	21°	21°
	Oxygen consumed, cubic centimeters per hour							
First hour normal	0.57	0.61	0.33	0.37	0.44	0.30	0.76	0.82
Second hour normal	0.43	0.45	0.31	0.30	0.38	0.38	0.76	0.81
Third hour normal	0.53	0.48			0.38	0.43	0.87	0.80
First hour KNC	0.13	0.13	0.09	0.13	0.15	0.14	0.29	0.28
Second hour KNC	0.10	0.11	0.07	0.08	0.11	0.13	0.17	0.44
Third hour KNC	0.13	0.10		0.09	0.09	0.08	0.21	0.11
Per cent decrease	77	78	75	69	71	70	72	66
					24 hours later			
Recovery					0.39	0.41		

cyanide produces its effect so rapidly that the respiration is decreased to its maximum extent by the end of the first hour exposure. In the dilute solutions, however, some little time is required for the depression

to reach its maximum value. This appears in tables 9 and 10, where the oxygen consumption during the first half hour in cyanide shows no depression. In fact, in some of these experiments, 35a, 35b, table 9,

TABLE 6

Experiments with 1/25000 mol. KNC

NUMBER OF EXPERIMENT	8a	8b	10a	10b	14a	14b	28a	28b
TEMPERATURE	23°	23°	22°	22°	23°	23°	21°	21°
	Oxygen consumed, cubic centimeters per hour							
First hour normal	0.40	0.51	0.35	0.32	0.50	0.49	0.67	0.74
Second hour normal	0.36	0.46		0.27	0.46	0.42	0.80	0.79
Third hour normal	0.38	0.46	0.42	0.32	0.44	0.46	0.70	0.79
First hour KNC	0.13	0.25			0.21	0.24	0.41	0.41
Second hour KNC	0.14	0.20	0.22	0.14	0.21	0.24	0.36	0.34
Third hour KNC	0.12	0.21	0.20	0.14	0.21	0.25	0.45	0.40
Per cent decrease	66	55	47	55	55	57	56	51
					After 24 hours		After 24 hours	
Recovery					0.41	0.43	0.79	0.81

TABLE 7

Experiments with 1/50000 mol. KNC

NUMBER OF EXPERIMENT	9a	9b	15a	15b	17a	17b	30a	30b
TEMPERATURE	22°	22°	22°	22°	23°	23°	21°	21°
	Oxygen consumed, cubic centimeters per hour							
First hour normal	0.44	0.33	0.37	0.39	0.42	0.44	0.74	0.66
Second hour normal	0.44	0.33	0.41	0.43	0.44	0.45	0.78	0.81
Third hour normal	0.44	0.32	0.41	0.42	0.43	0.43	0.78	0.79
First hour KNC	0.27	0.22	0.26	0.25	0.31	0.34	0.50	0.54
Second hour KNC	0.24	0.17	0.25	0.26	0.32	0.35	0.46	0.48
Third hour KNC	0.27	0.21	0.24	0.23	0.34	0.34	0.40	0.40
Per cent decrease	42	39	37	41	25	20	41	42
	3 days				24 hours		48 hours	
Recovery	0.39	0.30			0.45	0.46	0.67	0.78

and 21a, table 10, there are evidences of an acceleration of the rate of oxygen consumption during the first half-hour in cyanide. However, more experiments with these very dilute solutions would be required

to establish this point. Apparently the action of the cyanide is constant, that is, the respiration soon drops to a certain figure and stays there as long as the same concentration of cyanide is present. There

TABLE 8

Experiments with 1/100000 mol. KNC

NUMBER OF EXPERIMENT	16a	16b	19a	19b	23a	23b	31a	31b	32a	32b
TEMPERATURE	20°	20°	23°	23°	22°	22°	21°	21°	21°	21°
	Oxygen consumed, cubic centimeters per hour									
First hour normal	0.42	0.42	0.37	0.35	0.25	0.27	0.63	0.81	0.55	0.67
Second hour normal	0.48	0.46	0.39	0.40	0.32	0.30	0.67	0.80	0.65	0.67
Third hour normal	0.41	0.37	0.38	0.35	0.27	0.29	0.67	0.74	0.57	0.70
First hour KNC	0.37	0.38	0.37	0.36	0.23	0.24	0.54	0.62	0.50	0.59
Second hour KNC	0.34	0.36	0.29	0.27	0.23	0.20	0.52	0.58	0.40	0.49
Third hour KNC	0.32	0.34	0.33	0.29	0.22	0.21	0.52	0.58	0.43	0.47
Per cent decrease	21	14	13	17	20	24	20	25	27	25
							3 days		48 hours	
Recovery							0.59	0.68	0.56	0.64

TABLE 9

Experiments with 1/150000 mol. KNC

NUMBER OF EXPERIMENT	34a	34b	35a	35b
TEMPERATURE	20.5°	20.5°	20.5°	20.5°
	Oxygen consumed, cubic centimeters per hour			
First hour normal	0.54	0.54	0.54	0.54
Second hour normal	0.57	0.56	0.55	0.54
Third hour normal	0.49	0.49	0.59	0.55
First half-hour KNC	0.27	0.27	0.38	0.38
Second half-hour KNC	0.24	0.24	0.29	0.27
Second hour KNC	0.43	0.42	0.47	0.49
Third hour KNC	0.41	0.42	0.42	0.48
Per cent decrease	16	15	15	8
	24 hours		24 hours	
Recovery	0.56	0.54	0.52	0.53

is no cumulative effect of the cyanide. However, one would have to carry on the experiments longer than three hours to establish this point with certainty. It may again be emphasized that the decreased

oxygen consumption in cyanide is in no way related to muscular or ciliary activity, since the animals are perfectly quiet and motionless throughout the course of the experiments.

In a paper which accompanies this one, Child gives the results of his determinations of the carbon dioxide output of Planarians which have been exposed to cyanide. The carbon dioxide production was decreased after exposure to cyanide. That cyanide decreases the respiratory exchange in Planaria must therefore be regarded as an established fact, and conclusions which have been drawn by workers in this laboratory through the use of cyanide as a depressing agent thereby receive support of the most convincing kind.

TABLE 10

Experiments with 1/200000 mol. KNC

NUMBER OF EXPERIMENT	21a	21b	36a	36b
TEMPERATURE	22°	22°	20.5°	20.5°
	Oxygen consumed, cubic centimeters per hour			
First hour normal	0.30	0.35	0.48	0.50
Second hour normal	0.34	0.34	0.54	0.56
Third hour normal	0.29	0.32	0.56	0.51
First half-hour KNC	0.18	0.17	0.26	0.26
Second half-hour KNC	0.13	0.13	0.26	0.29
Second hour KNC	0.29	0.28	0.40	0.42
Third hour KNC	0.29	0.30	0.43	0.44
Per cent decrease	5	13	15	11
	24 hours			
Recovery	0.32	0.32		

SUMMARY

1. An extensive review of the literature on the chemical, physiological and pharmacological action of the cyanides is given. All of this literature supports the generally accepted opinion that the cyanides depress physiological processes in general, and rate of oxygen consumption in particular.

2. A large number of experiments are presented which prove that the oxygen consumption of Planaria is decreased in the presence of potassium cyanide. The amount of decrease depends upon the concentration of the cyanide, ranging from 80 to 90 per cent in 1/2000 mol.

KNC to 5 to 15 per cent in 1/200,000 mol. solutions. The decrease is independent of muscular or ciliary activity. It is entirely reversible, the animals being wholly uninjured by the cyanide and returning to their normal rate of oxygen consumption when the cyanide is washed out of them.

3. Conclusions drawn by workers in this laboratory from experiments on Planaria in which cyanide was used as a depressing agent and in which the results were interpreted on the basis that cyanide is a general protoplasmic depressant, therefore receive convincing support.

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THE EFFECT OF CYANIDES ON CARBON DIOXIDE PRODUCTION AND ON SUSCEPTIBILITY TO LACK OF OXYGEN IN PLANARIA DOROTOCEPHALA

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SUSCEPTIBILITY IN RELATION TO PHYSIOLOGICAL CONDITION

The following study of carbon dioxide production and susceptibility to lack of oxygen in the flatworm, *Planaria dorotocephala*, as influenced by cyanides is presented as a contribution to the problem of the relation between susceptibility and physiological or metabolic condition. Extensive studies on susceptibility to cyanides and to many other agents, including various anesthetics, acids, alkalies, salts, several alkaloids, "vital" dyes, H_2O_2 and certain physical conditions such as lack of oxygen and high and low temperatures, have been made on a great variety of organisms, including algae (11), (13), (14), protozoa (3), (19), *Planaria* (2), (4), (5), (7) and various other flatworms, developmental stages of starfish and sea-urchin (6), (9), (12) and annelids (16), (17) and data not yet published on various species of coelenterates, flatworms, echinoderms, arthropods and vertebrates. These studies on susceptibility have consisted in determining the survival time or the degree of inhibition of developmental or other processes in higher concentrations and the ability to become acclimated or acquire tolerance in lower concentrations, in relation to region of body (2), (3), (6), (8), (9), (11), (12), (13), (14), (16), (17), (19), physiological age and nutritive condition (4), (7), stimulation by various means (5), and the direction of functional conduction of stimuli (15). The existence of definite graded differences in susceptibility in relation to the physiological body-axes has been demonstrated for all axiate organisms examined, and it has been found that these susceptibility gradients correspond to characteristic differences in rate of development along an axis, and a corresponding gradient in protoplasmic structure is also very generally present in early developmental stages (8). These axial

susceptibility gradients also afford a basis for extensive control and modification of form and proportion in ontogeny (12), (16) and regulation (10), since the graded difference in susceptibility along an axis makes possible a graded inhibition, acceleration, acclimation or recovery along that axis. The experimental data already obtained constitute a rational physiological basis for the interpretation of many forms of teratological development.

In an earlier paper Dr. L. H. Hyman (17) suggested that a relation between the differences in electric potential which give rise to the bio-electric currents and the differences in susceptibility exists and recently Doctor Hyman and Mr. A. W. Bellamy have been able to show in this laboratory not only that this relation between regional differences in susceptibility and in electric potential actually does exist, but also that there is a relation between the chief susceptibility gradients and the galvanotactic reaction of animals, orientation occurring with the region or regions of highest susceptibility toward the cathode. The region of highest susceptibility is electro-negative through the galvanometer to regions of lower susceptibility, i.e., the region of highest susceptibility is electro-positive internally to other regions and in galvanotactic orientation this region is directed toward the cathode. Except for a brief article by Doctor Hyman in *Science* (27), these data on the relation between susceptibility gradients, galvanotaxis and bio-electric currents are as yet unpublished.

Certain minor differences appear in the susceptibility relations between different body-regions or organs, as determined by different agents or conditions, particularly in the later stages of development of the higher animals, where differentiation of different organs is relatively great. The important fact, however, is the very high degree of uniformity in susceptibility relations, particularly in the earlier stages of development and in the lower organisms, both plant and animal, to certain ranges of concentration or intensity of a great variety of agents. This fact indicates that the susceptibility of protoplasm to the action of external agents within certain limits of concentration or intensity is primarily an expression of some very fundamental factor in physiological condition.

It has been suggested by some that susceptibility depends merely on permeability of membrane and that the apparent differences in susceptibility are simply differences in permeability to the agents employed. While there can be no doubt that permeability is a factor in determining the relation between protoplasm and its environment,

permeability alone will not serve to account for the facts of susceptibility. In the first place, the regions of the body which are most susceptible to high concentrations or intensities are likewise able to acclimate or acquire tolerance most rapidly and most completely to a certain range of low concentrations or intensities (1), (2), (7, chap. iii), (12), i.e, not merely the penetration of the agent but the physiological activity of the living protoplasm is concerned in susceptibility. Second, the susceptibility relations, e.g., along an axis, to certain extreme temperatures and to lack of oxygen are the same as the susceptibility relations to chemical agents in certain concentrations. In the case of temperature and lack of oxygen the effect is obviously not a matter of penetration of a chemical substance through a membrane. And finally, it must be remembered that permeability of the plasma membrane itself is not a fixed condition established independently of other conditions in protoplasm, but changes with changes in protoplasmic conditions. It is becoming more and more evident that the plasma membrane consists of living protoplasm, and much recent work on permeability emphasizes the importance of chemical reaction in relation to permeability. (See for example (20, p. 443); (21, p. 368); (22, p. 366); (23)).

The high degree of uniformity and the absence of specificity in the general susceptibility relations to a great variety of agents, including cyanides, alcohol, ether, various urethanes, salts, acids, alkalies, alkaloids, vital dyes, temperature and lack of oxygen within certain limits of toxic concentration appears in various ways. For example the general susceptibility gradients along the body axes of the individual, as indicated either by the progress of death in lethal concentrations, by the degree and rapidity of acclimation or recovery in or after lower concentrations, or by the differential effect on the rate and course of embryonic development and regulation and on various functional activities, are essentially the same for all these agents and conditions. Similarly, differences in susceptibility in different individuals, corresponding to differences in physiological age, nutritive condition, etc., also show the same uniformity with the different agents and conditions. It is perhaps necessary to emphasize the fact that this uniformity in susceptibility relations does not in any sense imply that the action on living protoplasm of all these agents is of exactly the same sort at all concentrations or intensities. It is merely a statement of the fact that within certain limits of toxic concentration or intensity the regional and individual differences in susceptibility are

similar. Many agents, e.g., the anesthetics, produce in low concentration or with short period of action temporary, readily reversible effects, while with higher concentration or longer time the effects are less readily reversible or irreversible and finally end in death. So far as differences of this sort exist, it is the less readily reversible or irreversible changes which are primarily concerned in these general susceptibility relations, and the relations may or may not be the same for the readily reversible changes.

The action of benzamid will serve as an illustration of this point. Meyer (24) found that the minimal concentration of benzamid necessary to produce complete narcosis in tadpoles was $m/500$ at 3° and $m/200$ at 30°, and since the fat solubility of benzamid decreases with rising temperature, he suggested that the higher minimal narcotic concentration at the higher temperature was to be accounted for by the decrease in fat solubility. Experiments of my own with benzamid on *Planaria* (1, pp. 182–190) confirm those of Meyer as regards minimal narcotic concentration in relation to temperature, but with considerably higher concentrations which are lethal within 5 to 10 hours, the relation between susceptibility, concentration and temperature is reversed and death occurs earlier in a given concentration at high than at low temperature, i.e., susceptibility to these higher concentrations increases instead of decreasing with rise in temperature. This susceptibility to slowly lethal concentrations of benzamid shows the same relations as susceptibility to other agents.

Again, it is apparently an established fact that in certain low concentrations alkalies accelerate and acids retard protoplasmic activity, or more specifically oxidations, but in sufficient concentration both are toxic or lethal, and I have found that the regional and individual differences in susceptibility to such concentrations of acids and alkalies are in general similar and like those observed with other agents.

In short, whatever the particular method of action and effect on living protoplasm of an agent in concentrations whose effects are readily reversible, there is a high degree of uniformity in the susceptibility relations of protoplasm to higher, toxic or lethal concentrations of at least a very large number of agents, and these relations are essentially the same as those observed in injurious temperatures and in absence or insufficiency of oxygen.

In general, high susceptibility to concentrations and intensities which kill too rapidly to permit acclimation or the development of tolerance, is associated with a high capacity for acclimation or de-

velopment of tolerance to a certain range of lower concentration or intensities. Consequently, relative susceptibility can be determined in two ways: first, directly, by the survival time or the degree of inhibition or toxic effect in concentrations or intensities too extreme to permit acclimation or tolerance to develop; second, indirectly, by the capacity of the organism to acclimate or acquire tolerance in a certain range of lower concentrations or intensities. These two ranges of concentration or intensity must be determined experimentally for each agent or condition and for each species of organism, although it is in general true that for closely related species the two ranges of concentration of a given agent do not usually differ very widely.

It is evident from what has been said that the susceptibility relations when acclimation or tolerance are excluded are the opposite of those determined by means of acclimation or tolerance. For example, in all axiate organisms examined thus far, both plants and animals, the susceptibility as determined directly with the higher concentrations decreases primarily from the apical region or the head in the basal or posterior direction, i.e., inhibition, injury or death occur first in the apical or head region and progress in regular manner along the chief axis. In the range of lower concentrations, however, the apical or head region shows the greatest capacity for acclimation or tolerance and in the long run inhibition, injury or death appears first or most distinctly in the basal or posterior region and may be limited to that region or may progress a greater or less distance toward the apical or head region (see, for example, (2), (8), (11), (12), (13), (14)). In view of these facts it is necessary to distinguish between these opposite susceptibility relations. The one depends directly upon the condition of the protoplasm at the moment, the other upon the ability of the protoplasm to alter its condition in the course of time. The two modifications of the method of determining differences in susceptibility have commonly been referred to in the past as the direct and the indirect or acclimation method, since in the one the susceptibility is directly determined, in the other indirectly through the differential capacity for acclimation or tolerance.

I wish particularly to emphasize the fact of the association of these susceptibility relations with certain ranges of concentration or intensity of action of external agents. Investigation of the effects of external agents has been very largely concerned with the dissimilar effects of various low concentrations and intensities, the action of the more highly toxic and lethal concentrations being often regarded as of

less interest and importance. Consequently the fact of uniformity of susceptibility relations to a certain range of concentrations or intensities of at least a very large number of external agents and the physiological significance of these relations have not been clearly recognized. This fact is not in conflict with any data concerning the differences in the method of action of different agents or groups of agents within "physiological" concentrations or intensities, but merely indicates that, whatever the method of action in a particular case, a toxic or lethal effect may result with sufficiently high concentration of intensity and that the susceptibility relations to such toxic or lethal effects are, at least to a very high degree, uniform and independent of the particular agent employed, and are not necessarily the same as the susceptibility relations to its action within the limits of rapid reversibility. All the facts indicate that the more or less specific differences in action of different agents on protoplasm are primarily or chiefly associated with reversible or more readily reversible changes, and the uniformity in susceptibility relations with irreversible or less readily reversible changes. If this is true, then the range of concentration or intensity for which this uniformity holds is the range which produces such irreversible or less readily reversible changes in protoplasm.

Concentration or intensity of action of the agent is then of fundamental importance in determining susceptibility relations, and it is only by working with a wide range of concentrations or intensities, from those which kill almost immediately, to those which are non-toxic or merely "physiological" in their action, that any adequate conception of the relations between the protoplasmic system and agents which affect it can be attained. It has been possible to demonstrate more or less completely in various cases that the general susceptibility of protoplasm is related to its metabolic condition, or more specifically to the rate of certain fundamental metabolic reactions, probably primarily the oxidation or certain oxidations. For example, in *Planaria* motor activity increases the direct susceptibility of the worms to cyanides and other agents in toxic concentration (1, pp. 167–170). This experiment I have used extensively as a class experiment. Other forms of stimulation, e.g., section of the body, have also been found to increase both direct susceptibility and CO_2 production (5, p. 437); moreover, direct susceptibility, oxygen consumption and CO_2 production all decrease with advancing physiological age (7). On the basis of these facts alone, these differences in susceptibility might be accounted for

by differences in permeability of membranes, but when we find that both direct and indirect susceptibility to temperature and susceptibility to lack of oxygen show the same differences in relation to region of body, physiological age, nutritive condition, etc., as direct and indirect susceptibility to cyanides and other chemical agents, it is evident that we are concerned not merely with permeability as a physical condition but with fundamental activities of protoplasm. Moreover, the fact that the susceptibility relations to low oxygen or lack of oxygen are the same as the direct relations to other agents, indicate very clearly that susceptibility in the sense in which the word is used here is in some way connected with the oxidations or with certain oxidations. And finally, the fact that these susceptibility relations are not specific, but rather essentially identical, showing only slight quantitative differences with a very wide variety of substances and conditions in proper concentration or intensity, indicates that the factor fundamentally concerned is not qualitative and specific, but quantitative.

Bearing all the facts in mind, the simplest and most satisfactory statement at present is that susceptibility is associated with or dependent upon rate of oxidations or of certain oxidations in protoplasm. While I have made this statement repeatedly, I have likewise endeavored to point out that it must not be interpreted as meaning that all agents and conditions act directly or in any specific way upon the oxidative reactions, or even that all act in the same way on protoplasm. But whether a particular agent enters chemically into the oxidative reaction, acts as a reducing agent, affects the oxidizing enzymes or alters the aggregate condition of the protoplasm colloids in some way, susceptibility to that agent within certain limits of concentration may still be dependent on and a measure of the relative rates of oxidation in different body-regions and in different conditions of age, nutrition, activity, etc. This is all that such a statement can mean at present, though in future, as we learn more of the action of each particular agent on protoplasm, we shall doubtless be able to state more definitely the nature of the relation between the action of each agent and the rate of oxidation. At present, however, it is scarcely possible to say more than that the rate of oxidation is dependent upon a complex of factors in living protoplasm and that each such factor may play a rôle both as an index and a determiner of oxidation rate.

The biochemist has frequently said that such expressions as "rate of metabolism" and "rate of oxidation" have no value or meaning.

From a certain biochemical viewpoint which conceives each particular chemical reaction as a discrete entity, this statement is unquestionably correct. But it is just as certain that from a certain physiological viewpoint these expressions possess a very real significance and are at present necessary for statement of the experimental data. The general relation between susceptibility and oxidation rate is an experimental fact, and the statement of this relation is merely the first step toward its analysis. The probability that if we knew more about the matter we might be able to state the relation in more definite terms for each particular case, does not lessen the value of the general statement as a starting point. Living protoplasm is a system of physiologically correlated and interdependent changes and conditions, not a mosaic of wholly independent reactions. When we examine this system in its chemical aspects, the oxidations appear as highly significant or fundamental factors, while in another aspect the active mass of enzymes, colloid aggregation or permeability of membranes may be regarded as fundamental. But all such factors are unquestionably more or less closely correlated and it is the same system in all cases. With certain limitations we can use the rate of oxidation in such a protoplasmic system as a measure of its physiological or vital condition, and, so far as this is possible, the conception is justified. The relation between susceptibility and rate of oxidation is simply this relation between rate of oxidation and physiological condition stated in terms of the action of external agents. Like any other general statement of relation it is subject to modification and limitation and may give place to more definite statements as experiment advances our knowledge, but as far as it goes, it is a statement of fact.

This relation may be stated in the following terms: The direct susceptibility, i.e., susceptibility to the agents employed in sufficient concentration to prevent acclimation or acquirement of tolerance, but not immediately lethal, varies in general directly, though not necessarily proportionally with the rate of oxidation (1), (4), (7, chap. iii). The indirect susceptibility associated with ability to acclimate or acquire tolerance to a certain range of low concentrations and intensities of the agents employed, varies inversely though not necessarily proportionally with rate of oxidation.

To my mind these susceptibility relations are simply one expression of the fact that living protoplasm is a system of interdependent and correlated conditions and changes, in which dynamic equilibration is continuously going on and new disturbances are continuously oc-

curring. Since regional and individual differences in the condition of this system exist, there must also exist regional and individual differences in susceptibility to all agents which alter to a sufficient degree any of the correlated factors constituting the system. Moreover, these differences in susceptibility must show a certain definite and in general uniform relation to certain fundamental factors in the system. Such, I believe, is the relation between susceptibility and oxidation rate; not in any sense specific, not necessarily direct, undoubtedly to be stated in different terms in different cases, and meaning merely that rate of oxidation is associated with and dependent on various factors in the protoplasmic system which are susceptible to alteration by external agents.

No claim is made for universality of the relations as stated, though they have been experimentally demonstrated for a large number of agents and organisms (pp. 372 to 373). It remains to determine their limitations, their nature in particular cases and their value as a method of investigation. As regards the latter point, their value as a method for attacking various problems has already been abundantly demonstrated. The susceptibility method, checked in various ways, has served not only for demonstrating physiological axes in plants (11), (13), (14), protozoa (3) and various other animals from nearly all the great groups, but it has made possible the control and modification of development and a rational interpretation of various types of teratology (12), (16) and experimental data to be published later will show that it has given us a basis for interpretation of galvanotactic reactions and bio-electric currents. Moreover, it has been an important factor in the development of a conception of physiological polarity, axiation and integration which has received experimental support and confirmation from many different sources (8). Practically it has amply justified itself.

In work with this susceptibility method the cyanides have proved to be very useful agents, particularly in the direct determination of susceptibility, because of their high toxicity and the very slow and slight acclimation to their action. Moreover, various lines of evidence which are discussed by Doctor Hyman in the paper appearing in this number of this Journal, show very clearly that the cyanides do act in some way on the rate of oxidation. As regards the general susceptibility relations, the differences between cyanides and other agents are merely quantitative. Salts of the heavy metals, e.g., $HgCl_2$, $CuSO_4$, etc., can be used in even greater dilution than the cyanides for susceptibility determination and the same regional and individual differ-

ences appear in survival time, rate of development and form and proportion as with cyanides, the chief difference observed being that acclimation or tolerance to the heavy metal salts develops somewhat more rapidly and to a greater degree than to cyanides.

Cyanides are not used as "specific" agents for determination of susceptibility, as certain critics of the method have apparently believed, but merely as convenient agents, because they act in low concentration and so permit the exclusion of osmotic or other effects associated with high concentration and because the very slow and slight acclimation makes it possible to distinguish sharply between direct and indirect or acclimation susceptibility. Whatever the nature of the relation between the toxicity of cyanides and the protoplasmic oxidations, much evidence has accumulated to show that cyanides decrease oxidation in some way, although in some cases a primary stimulation occurs with extremely low concentration. For all these reasons the cyanides have been much used in the work on susceptibility, but essentially the same results may be obtained with many other agents.

The most extensive studies on susceptibility in relation to different body regions, to differences in physiological age, nutritive conditions, temperature, etc., have been made with the flatworm *Planaria dorotocephala* as material (1), (2), (4), (5), (7), (10). Since the cyanides have been much used in this work and for other forms as well, it seems desirable, even though the results obtained have already been checked by CO_2 estimation with the Tashiro biometer (1), (7, chap. iii) and in various other ways, to present additional data concerning the effect of cyanides on oxidation in this species. From what we already know, there can be little doubt that cyanides decrease oxidation at least very generally in organisms and the evidence already at hand indicates very clearly that they act in this way on *Planaria*. Nevertheless, since so much work has been done with *Planaria* as material and the cyanides as agents it seems worth while to make the demonstration as complete as possible. Such a demonstration serves to throw some light not only on the relation between susceptibility to cyanides and rate of oxidation, but on the whole problem of susceptibility to agents in general.

Dr. Hyman's paper presents the data on the effect of cyanide on oxygen consumption in *Planaria*, as determined by the Winkler method, and the following sections of the present paper give some supplementary data on the effect of cyanide on CO_2 production, estimated colorimetrically, and on susceptibility to lack of oxygen, determined by survival time.

THE EFFECT OF CYANIDE ON THE PRODUCTION OF CARBON DIOXIDE

In these determinations the colorimetric method with phenolsulphonephthalein as indicator was employed, the Hynson, Westcott & Dunning set of colors in buffer solutions being used as the standard for comparison. For each experiment worms from the same stock, of the same size and nutritive conditions are used in two lots of as nearly as possible equal weights. In nearly all experiments the heads are removed before weighing in order to prevent as far as possible motor activity, which in animals with heads may be sufficient to increase the CO_2 production to a considerable extent. It has been determined that removal of the heads has no immediate effect on CO_2 production except a slight temporary increase during the first few hours after decapitation. The heads are of course regenerated, and after two days or more there is an increase in CO_2 production connected with the development of the embryonic tissue which gives rise to the new head, but within the first forty-eight hours after section this is very slight or inappreciable as compared with the total and the animals were in most cases used within this period. Moreover, since the number of worms in the two lots to be compared is equal or nearly equal, the number of regenerating regions is the same or nearly the same in both, and the differences in CO_2 production from this source are negligible.

The animals are weighed dry, i.e., they are placed on filter paper to remove excess water before weighing and then transferred on the blade of a small lancet to a small glass container already balanced and standing on the balance pan. Weighing is done as rapidly as possible for injury results from exposure to the air for more than one or two minutes. As soon as weighed the animals are returned to water, and then in some cases a preliminary comparison of CO_2 production in the two lots is made before subjecting one of them to the action of KNC.

For the colorimetric determination of CO_2 pyrex glass tubes of 5 to 6 cc. volume and of the same diameter as the standard tubes are used, the worms being placed in these tubes in equal volume of phenolsulphonephthalein solution made up to give the same density of color as the colorimeter tubes. The open end of the tube is first closed with a small plug of thoroughly washed absorbent cotton soaked in the same indicator solution, air bubbles being excluded, and is then sealed with melted paraffine. This method of closure has been found preferable for these animals to the rubber tubing and clamp used by Haas (25), because even the headless animals may creep about to

some extent during the first few moments of the experiment, and if rubber tubing and clamp are used they tend to come to rest in the rubber tubing, where the light intensity is least. There they are out of sight and differences in motor activity cannot be observed, moreover they are often crushed or lost in removal. With the cotton-paraffine closure the cotton serves for the usual period of an experiment as an inner seal.

The CO_2 production is determined in terms of pH, the minus logarithm of the hydrogen ion concentration, by matching at intervals the tubes containing the worms with the standard tubes of known pH. That the animals do not produce any non-volatile acids in appreciable quantities is demonstrated by the fact that after change in color of the indicator by the worms, shaking with air restores the original color, and the probability that any other volatile acid than carbonic acid is produced is negligible.

The preliminary pH determination usually extends over two or three hours with half-hourly or hourly readings of pH. At the end of this time the animals are removed from the tubes, one lot being placed in water, the other in KNC of the concentration used in that particular experiment. The animals remain in KNC for various lengths of time, but before death and disintegration of any part begin (in a few cases with the longer periods in KNC very slight traces of disintegration were observed), they are returned again to water, washed in several changes of water and allowed to remain from 5 to 30 minutes before being placed in the indicator solution. After this time both the cyanide lot and the control are again placed in clean tubes with fresh indicator solution, the tubes sealed and the pH determined half-hourly or hourly or at longer intervals for several hours. With some lots the experiment was concluded at this point, but with others observations were made on recovery, the two lots being again returned to water and their CO_2 production being again compared after 24 or 48 hours. In many experiments then there are three comparative estimations of CO_2 production, first preliminary, second immediately after KNC, third during or after recovery.

The chief disadvantage of this method is that the CO_2 estimation is made after removal of the animals from cyanide rather than in its presence. The KNC solution is of course alkaline and its alkalinity changes more or less on standing, so that it is difficult to bring it to the same pH as the water used in the control. The effect of cyanide determined in this way is then merely the effect that remains after

return of the animals to water. The animals begin to recover during the CO_2 estimation, and partial recovery sometimes appears in the later stages of the estimation. This method serves merely to demonstrate that CO_2 production is affected by cyanides, but does not give quantitative data. The determinations of the effect of cyanides on oxygen consumption in Doctor Hyman's paper give much more striking results than those on CO_2 production, because the oxygen consumption is determined in the presence of cyanide, not merely after its action.

The water used in all experiments is a well water, the same in which the stocks of worms are kept, the tap water being unavailable because of chlorination. When this water is first pumped pH = 7.6, but on standing a small amount of CO_2 is given off and the pH rises to 7.8 or even to 8.0. This accounts for the fact that in the following tables the pH at the beginning of different experiments is different. Since the relative CO_2 production of cyanide and control lots is of interest here rather than the absolute CO_2 production, the pH at the beginning of the experiment need not always be the same in different experiments, but is always the same for experimental and control lots in any one experiment. The results are given in terms of pH, the decrease in pH representing the increase in hydrogen ion concentration resulting from CO_2 production in the respiration of the animals. In the pH data as given in the table the figures in the first decimal place are obtained directly by matching with the standard tubes, while those of the second place are based on estimation from the two standard tubes between which the color of the experimental tube lies.

The chief data are presented in tables 1 to 4. The first four vertical columns in tables 1 and 4, the first three columns in tables 2 and 3 give the general data of each series, series number, length in milligrams of animals used where the table includes worms of different length, the number of worms in each lot and the weights of each lot in fractions of a gram. The remaining columns have to do with the data of each particular experiment. In most series the data are divided into three groups as indicated in the column headed experimental conditions: first, preliminary, where both lots A and B are in water; second, KNC, where lot A has been in KNC of the concentration and for the time indicated; third, recovery, in which the animals of lot A have been in water a certain number of hours as indicated in each experiment since treatment with KNC. In a few series, e.g., series 680 in table 1, no observations were made on recovery, while in series 667 II in table 2 two different periods of recovery are recorded, but no preliminary

observations were made. The columns giving the pH represent half-hour or hour intervals, but the actual intervals between observations differ as indicated in different series.

The important point in each series is the difference in pH which appears during two to four hours between the cyanide lot A after exposure to KNC and the control lot B, as compared with the difference

TABLE 1

Well-fed worms 16 to 25 mm. KNC *m/1000*

SERIES	LENGTH	NUMBER OF WORMS	WEIGHTS	EXPERIMENTAL CONDITIONS	pH AT HALF-HOUR INTERVALS					
					0	½	1	1½	2	3
	mm.		*grams*							
680 A	25	5	0.1064	Preliminary A. water	7.6	7.5	7.33	7.25	7.2	
680 B	25	5	0.1043	Preliminary B. water	7.6	7.5	7.33	7.25	7.2	
				KNC A. KNC 3 hours	7.6	7.43			7.27	
				KNC B. water	7.6	7.4			7.22	
				Preliminary A. water	7.7	7.57	7.5	7.4	7.3	
				Preliminary B. water	7.7	7.6	7.55	7.43	7.33	
696 A	16	8	0.08	KNC A. KNC 3 hours	7.75	7.6	7.5		7.35	7.25
696 B	16	8	0.0757	KNC B. water	7.75	7.53	7.4		7.27	7.17
				Recovery 48 hours A. water	7.77		7.43		7.25	
				Recovery 48 hours B. water	7.77		7.43		7.25	
				Preliminary A. water	7.77	7.6	7.53			7.2
				Preliminary B. water	7.77	7.63	7.55			7.23
692 A	16–18	8	0.0865	KNC A. KNC 5½ hours	7.6	7.5			7.3	
692 B	16–18	8	0.0814	KNC B. water	7.6	7.43			7.23	
				Recovery 43¼ hours A. water	7.7	7.55	7.4	7.35		7.13
				Recovery 43¼ hours B. water	7.7	7.55	7.43	7.37		7.15

between these two lots in the preliminary and the recovery determinations. It will be observed that in every series the decrease in pH in lot A, the lot exposed to KNC *is less rapid after exposure as compared with that in lot B* than in either the preliminary or the recovery determinations. This difference is not due to difference in weight in any case, for not only are the differences in weight between the two

TABLE 2

Well-fed worms 8 to 10 mm. KNC *m/1000*

SERIES	NUMBER OF WORMS	WEIGHTS	EXPERIMENTAL CONDITIONS	pH AT HOURS							
				0	½	1	1½	2	2½	3	4
		grams									
			KNC A. KNC ½ hour	7.75		7.73				7.7	7.63
			KNC B. water	7.75		7.7				7.6	7.5
667 II A	4	0.0131	Recovery 22 hours A. water	7.75				7.7			7.65
667 II B	3	0.0138	Recovery 22 hours B. water	7.75				7.67			7.55
			Recovery 72 hours A. water	7.77		7.75					7.63
			Recovery 72 hours B. water	7.77		7.73					7.6
			Preliminary A.water	7.9	7.8	7.73	7.63	7.53			
			Preliminary B.water	7.9	7.8	7.73	7.63	7.53			
679 A	11	0.0364									
679 B	10	0.0364									
			KNC A. KNC 1 hour	7.9				7.58		7.47	
			KNC B. water	7.9				7.53		7.4	
			Preliminary A.water	7.6	7.55	7.47	7.43	7.38			
			Preliminary B.water	7.6	7.55	7.47	7.43	7.38			
681 A	11	0.0333	KNC A. KNC 1 hour	7.6	7.57	7.53	7.48	7.43		7.35	
681 B	10	0.0315	KNC B. water	7.6	7.55	7.5	7.43	7.37		7.3	
			Recovery 42 hours A. water	7.7		7.65	7.5	7.4			
			Recovery 42 hours B. water	7.7		7.6	7.5	7.4			
			Preliminary A.water	7.65	7.55	7.47	7.4				
			Preliminary B.water	7.65	7.55	7.45	7.4				
683 A	10	0.0433									
683 B	10	0.0415	KNC A. KNC 2 hours	7.7	7.67	7.58	7.5			7.4	
			KNC B. water	7.7	7.6	7.5	7.4			7.3	
			Recovery 25 hours A. water	7.77	7.67				7.4		
			Recovery 25 hours B. water	7.77	7.7				7.42		

lots A and B of a pair slight, but in all cases the preliminary determinations show almost the same or exactly the same rate of change in pH in A and B, and in all series except 667 II in table 2 and 686 in

table 3, where there is any difference in weight between lots A and B, A is the heavier and should therefore produce more CO_2 than B. After a certain length of time in KNC, however, A produces less CO_2 than B. Differences in motor activities do not account for this difference, for in most cases the headless animals showed little or no motor

TABLE 3

Worms starved 51 to 53 days: 8 to 10 mm. KNC *m/1000*

SERIES	NUMBER OF WORMS	WEIGHTS	EXPERIMENTAL CONDITIONS	pH AT HOURS							
				0	½	1	1½	2	2½	3	3½	4
		grams										
			Preliminary A. water	7.8		7.75	7.7			7.6		
			Preliminary B. water	7.8		7.75	7.7			7.62		
686 A	8	0.0164										
686 B	8	0.0168										
			KNC A. KNC 2 hours	7.6				7.53		7.47		
			KNC B. water	7.6				7.5		7.43		
			Preliminary A. water	7.8	7.7	7.6	7.55		7.4			
			Preliminary B. water	7.8	7.72	7.62	7.58		7.43			
687 A	9	0.0451										
687 B	8	0.0444										
			KNC A. KNC 1 hour	7.6			7.43		7.37		7.3	
			KNC B. water	7.6			7.4		7.33		7.23	
			Recovery 19 hours A. water	7.77		7.62		7.4				7.3
			Recovery 19 hours B. water	7.77		7.62		7.42				7.3
			Preliminary A. water	7.7		7.6	7.55	7.47				
			Preliminary B. water	7.7		7.6	7.55	7.47				
684 A	8	0.0333										
684 B	8	0.0332										
			KNC A. KNC 1¼ hour	7.77		7.7				7.52		7.37
			KNC B. water	7.77		7.65				7.4		7.27
			Recovery 43 hours A. water	7.67		7.6		7.38				7.3
			Recovery 43 hours B. water	7.67		7.6		7.4				7.33

activity during the determinations, and all results in which differences in motor activity were sufficient to have constituted a possible factor in the pH differences are excluded from the tables. There is no escape from the conclusion that the difference in rate of change of pH after KNC is due to a difference in rate of CO_2 production which does not

TABLE 4

Well-fed worms 16 to 25 mm. KNC *various concentrations*

SERIES	LENGTH	NUMBER OF WORMS	WEIGHTS	EXPERIMENTAL CONDITIONS	pH AT HOURS							
					0	½	1	1½	2	2½	3	4
	mm.		*grams*									
				Prelimi- A. water	7.65	7.45	7.3	7.2	7.15			
				nary B. water	7.65	7.47	7.32	7.22	7.17			
682 A	25	5	0.1187	KNC A. KNC								
682 B	25	5	0.1152	*m*/500 2¾ hours	7.7	7.57	7.4					
				B. water	7.7	7.52	7.32					
				Recovery A.water	7.77	7.5				7.2		
				24 hours B.water	7.77	7.5				7.2		
				Prelimi- A. water	7.7	7.57	7.37	7.3				
				nary B. water	7.7	7.57	7.35	7.28				
689 A	25	6	0.1304									
689 B	25	6	0.1285	KNC A. KNC								
				m/2000 5 hrs.	7.75	7.5				7.23		
				B. water	7.75	7.45				7.17		
				Prelimi- A. water	7.75	7.53	7.38					
				nary B. water	7.75	7.55	7.4					
691 A	18–20	7	0.086									
691 B	18–20	6	0.0846	KNC A. KNC								
				m/10000 22½ hrs	7.6	7.4	7.33					6.87
				B. water	7.6	7.37	7.27					6.8
				Recovery A.water	7.8	7.6	7.4	7.33	7.27			
				45 hours B.water	7.8	7.6	7.4	7.32	7.27			
				Prelimi- A. water	7.6	7.4		7.2				
				nary B. water	7.6	7.43		7.2				
688 A	25	9	0.2253									
688 B	25	9	0.2182	KNC A. KNC								
				m/20000 61½ hrs.	7.7	7.5	7.25					
				B. water	7.7	7.47	7.2					
				Prelimi- A. water	7.77	7.67				7.3		
				nary B. water	7.77	7.67				7.32		
693 A	16–18	9	0.094									
693 B	16–18	8	0.0923	KNC A. KNC								
				m/25000 21½ hrs								
				hours	7.6	7.35			7.17			
				B. water	7.6	7.32			7.1			
				Recovery A.water	7.7	7.6	7.45				7.23	
				24 hours B.water	7.7	7.63	7.5				7.27	

exist in the preliminary nor in the recovery determinations to any such degree. Carbon dioxide production is unquestionably decreased by KNC.

In all series of tables 1, 2 and 3, KNC $m/1000$ was used. this being the concentration commonly employed for direct susceptibility determinations, but table 4 shows the effects of various concentrations ranging from $m/500$ to $m/25000$ with various periods of exposure.

A comparison of the preliminary, the recovery and the cyanide determinations shows in some cases that the absolute decrease in pH is more rapid in both A and B in the cyanide than in the preliminary or the recovery determinations. Series 693, table 4 is the most extreme case of this sort. Differences in temperature constitute the chief factor in determining differences of this kind. Most of this work was done during the winter months and the range of variation in the laboratory temperature was about 5°C. This range of variation may affect CO_2 production in different determinations, but in any one determination temperature conditions for the two lots are of course the same. Stimulation resulting from the necessary handling may also be concerned in some cases. In the KNC determination both the cyanide lot and the control are handled as nearly as possible in the same way, the control being washed as frequently as the KNC lot. These washings, together with the transfer to the tubes in pipettes, may cause a considerable degree of excitation which does not necessarily appear in increased motor activity, but does increase the rate of pH change during the first half-hour or so.

A considerable number of series not included in the tables was run without preliminary pH determination, but in all such cases the decrease in pH was less rapid in the KNC lot than in the control. Among all determinations made, only one, with KNC $m/10000$. showed a slightly higher rate of pH decrease in the KNC lot than in the control. This low concentration of KNC has but little inhibiting effect, except when its action is continued for a day or more, and in this particular case the KNC lot showed a greater motor activity than the control.

All determinations of table 1 were made with large, physiologically old worms, 16 to 25 mm. in length, while in those of table 2 the worms were considerably younger, only 8 to 10 mm. in length. A comparison of tables 1 and 2 shows that the smaller, physiologically younger worms are more susceptible to KNC than the larger, older animals, although no exact expression of this difference is obtainable from the data. The tables show merely that the retardation in the rate of change of

pH in KNC $m/1000$ in the younger animals is as great as, or greater than that in the older, with shorter times of exposure to KNC and although the total weights of the lots of young worms are only one-half to one-tenth those of the old. The differences in susceptibility indicated are in complete agreement with those previously observed in relation to physiological age (4), (7). Moreover, the CO_2 production, as indicated by pH, of the worms of table 3 which have been starved about 50 days is much more nearly like that of the young animals of table 2 than that of the older animals of table 1, a fact which also agrees, as might be expected, with the results of the direct susceptibility method.

In table 4, where the results for very different concentrations are given, only a few characteristic records are selected from among a larger number obtained. It is evident that with decrease in concentration a very great increase in time of exposure is possible without death. In the concentrations used in series 688 and 693, viz., $m/20000$ and $m/25000$, there is probably some degree of acclimation. The effect of $m/20000$ is no greater after $61\frac{1}{2}$ hours in series 688 than of $m/25000$ after $21\frac{3}{4}$ hours in series 693, and I have found in other experiments that some degree of acclimation may occur in these concentrations.

In the recovery determinations it was not infrequently observed that the cyanide lot A showed a higher CO_2 production in relation to the control than in the preliminary determinations. Series 683, table 2 and 693, table 4, show this condition. These results suggest the occurrence of a period of supernormal CO_2 production in the course of recovery, but further data are necessary to establish this point.

The attempt was made with very low concentrations and short periods of exposure to determine whether a stimulating effect, such as has been observed with low concentrations of cyanides in various other cases, could be detected. Animals in KNC $m/100{,}000$ for $\frac{1}{4}$, $\frac{1}{2}$ or 1 hour and those in $m/50000$ for $\frac{1}{4}$, $\frac{1}{2}$ or $1\frac{1}{2}$ hour showed no appreciable effect, either of stimulation or depression. After 3 hours in KNC $m/50000$ or $\frac{3}{4}$ hour in $m/20000$ there was very slight but distinct decrease in CO_2 production, as indicated by pH, extending over at least 2 hours. In these, as in the series with higher concentrations, the pH was determined after removal from the cyanide.

In another group of experiments the cyanide was made up in the indicator solution and brought as nearly as possible to the same pH as the water by the addition of HCl $m/1000$ and the CO_2 production in this solution was compared with that of the control in water and

with that of a preliminary determination. In these experiments where CO_2 production was estimated in the presence of KNC concentrations down to $m/250000$ showed apparently a distinct inhibiting action and even in $m/500000$ some slight inhibition seemed to occur. These data are perhaps not very conclusive because of the difficulty of bringing the KNC solution to the same pH as the water and because of the possibility of change in alkalinity in the solution itself. No indication of stimulation was observed in any case.

THE EFFECT OF CYANIDE ON SUSCEPTIBILITY TO LACK OF OXYGEN

For determining susceptibility to lack of oxygen water from which the oxygen has been largely removed by boiling or by other means is used. The well water used in the laboratory has so low an oxygen content before aeration, analysis giving only a trace of oxygen, that it can be used directly for this purpose. Susceptibility to lack of oxygen is determined by observing the survival times of animals in small volumes of low oxygen water, sealed so as to avoid entrance of air. In my experiments the same pyrex tubes were used as for the CO_2 experiments, but with only about 2 cc. of water. Since relative rather than absolute susceptibility is of interest for present purposes, two lots of individuals to be compared, consisting of three or four individuals each, may be placed in the same tube and so be under identical conditions as regards oxygen and CO_2. If the animals of the two lots are not readily distinguishable by size or other external characteristics, one lot may be marked, e.g., by cutting off a bit of the posterior end. This slight injury has no appreciable effect on the total susceptibility, but if we mark the lot expected to show the lower susceptibility of two to be compared, any stimulation which might result will only decrease the difference in susceptibility between this and the other lot.

The method of introducing the two lots into the same tube makes weighing unnecessary, for it makes no difference whether the two lots are of equal weight or not. It is of course also possible to compare two lots of equal weight in different tubes with equal volumes of water of the same oxygen content, but the possibilities of error are greater with this method.

In order to make certain that it is lack of oxygen and not accumulation of CO_2 that kills, sufficient NaOH may be added to make NaOH $m/1000$. This concentration is well below the toxic limit and prevents

too great acidity by the CO_2 produced. By adding also a little phenolsulphonephthalein to such a solution it is found that the animals almost always die from lack of oxygen while the solution is still alkaline, often while the alkalinity is pH 8.2 or more, i.e., near or beyond the limit of this particular indicator on the alkaline side. It is perfectly clear that death in such cases results from lack of oxygen, not from CO_2. Moreover, by introducing more or less oxygen into such a solution life may be prolonged as desired or recovery may be brought about after partial death and disintegration.

The first visible effect of lack of oxygen in *Planaria* is an increased negative geotaxis, then follows a decrease and loss, beginning in the head region, of the ability to attach to the substratum and creep and of muscular activity in general. Sooner or later the animals become completely inactive and lie more or less completely relaxed on the bottom, and death and disintegration follow. These changes occur first in the head region and in the posterior zoöids and from the head they progress posteriorly along the first zoöid. In other words, the gradient of susceptibility to lack of oxygen is the same as that to the higher concentrations of KNC and other agents (1), (2), (3), (4), (6), (9), (17), (19). Disintegration of the head and the posterior zoöids may occur while other regions are still alive and capable of recovery. In *Planaria* disintegration follows death almost at once or perhaps may precede death of many of the cells, and may be used as a convenient criterion of death.

There can be no doubt that this susceptibility to lack of oxygen is a more or less exact measure of oxidative metabolism and the fact, to which attention has already been called, that it shows the same regional and individual differences as direct susceptibility to other agents is significant evidence for the general relation between susceptibility and oxidative metabolism.

In a part of my experiments on the effect of cyanide on susceptibility to lack of oxygen the control and the cyanide lot after exposure to cyanide were placed in the same tube in low oxygen water. In others lots of equal weight were placed in different tubes in equal volumes of water, and in still others KNC was added to the low oxygen water in one tube and the susceptibility in this solution compared with that of a lot of equal weight in water. Only the low concentrations of KNC can be used in this way because the higher concentrations kill the animals before they are affected by lack of oxygen. Since animals affected by cyanide to any great degree show less motor activity than normal animals, the heads of all worms were removed.

The data for six series are given in table 5 in general form, without details of the progress of death and disintegration. Other series are essentially similar with similar results to some one of these six.

In the first three series of the table the animals of the experimental lot were placed in KNC for three or four hours and removed to water before the susceptibility determination, as indicated by "preceding" in the fourth column of the table, and KNC and control lots were placed in the same tube. In the last three, lots of equal weight were used and the KNC was added to the low oxygen water of the one tube of the pair and so was present "during" the susceptibility determination, KNC lot and control being in separate tubes.

In every series except 672 the susceptibility of the experimental (KNC) lot is distinctly greater, with the higher concentrations much

TABLE 5

Effect of KNC on susceptibility to lack of oxygen

SERIES	LENGTH	CONCENTRATION OF KNC	EXPERIMENTAL LOT IN KNC	SUSCEPTIBILITY
	mm.			
671	25	*m*/1000	3 hours preceding	Experimental lot > Control
670	25	*m*/1000	4 hours preceding	Experimental lot > Control
673	25	*m*/1000	4 hours preceding	Experimental lot > Control
675	25	*m*/10000	During	Experimental lot > Control
676	8–10	*m*/20000	During	Experimental lot > Control
672	25	*m*/50000	During	Experimental lot $\lesssim$ Control

greater than that of the control. In series 672 the susceptibility of the control was at first slightly greater, but in later stages all individuals of the cyanide lot were distinctly more susceptible. Whether this unusual result was due to the low concentration of the KNC or to other incidental factors could not be determined.

Animals in KNC, or that have been in KNC, are then more susceptible to lack of oxygen than normal animals. Since we know from other experiments that increase in rate of oxidation increases susceptibility to lack of oxygen, these results may appear at first glance to indicate that KNC increases the rate of oxidation. Such a conclusion, however, is directly opposed to all that we know in general concerning the action of the higher concentrations of cyanides on protoplasm. Not only Doctor Hyman's results on oxygen consumption and my own on CO_2 production, but many other experimental facts indicate very

clearly that the cyanides in some way decrease oxidation. How then shall we interpret these data on susceptibility to lack of oxygen?

In the light of all the known facts there is every reason to believe that living protoplasm subjected to the action of cyanides is in much the same condition as when subjected to lack of oxygen, i.e., the cyanides prevent or retard in some way the oxidative reactions or certain of them in such a way that their effect is additive to that of lack of oxygen. If this is the case, the lack of oxygen in my experiments continues or is added to the action begun by KNC, consequently the animals subjected to cyanides are more susceptible to lack of oxygen than the controls.

This fact, that cyanides increase susceptibility to lack of oxygen, constitutes, I believe, still further evidence in support of the conclusion that cyanides inhibit oxidation in some way and it agrees with the conclusion reached by Geppert (26) in suggesting that this action consists in preventing the tissues from obtaining or from utilizing oxygen which is present. Such an effect may be due to destruction or inactivation of oxidizing enzymes or to action as a reducing agent or to some other unknown method of action.

CONCLUSION AND SUMMARY

The results presented above are far less striking than Doctor Hyman's data on the effect of cyanides on oxygen consumption, and are to be regarded as merely supplementary to them. Doctor Hyman has measured oxygen consumption in the presence of cyanides while I have been concerned almost wholly with what remains of the effect after return of the animals to water. While all the data agree as regards the effects of KNC, they permit only certain general conclusions concerning quantitative differences in susceptibility, but these so far as they go, are in complete agreement with the conclusions drawn from experiments of other sorts.

The points of chief importance are summarized as follows:

1. A colorimetric method with phenolsulphonephthalein as indicator can be readily used for the comparative estimation of CO_2 production in terms of pH in *Planaria dorotocephala*. Differences in CO_2 production with physiological age, nutritive condition, etc., are easily demonstrated by this method.

2. KNC in concentrations ranging from $m/500$ to $m/25000$ and with periods of action from $\frac{1}{2}$ to $61\frac{1}{2}$ hours decreases CO_2 production in *Planaria dorotocephala*.

3. If the exposure to KNC is not too long, gradual recovery in CO_2 production occurs, apparently with a supernormal stage near the end of the recovery period.

4. Susceptibility to lack of oxygen in *Planaria* shows in general the same regional and individual differences as susceptibility to cyanides and other toxic agents. KNC increases susceptibility to lack of oxygen, i.e., an animal which has been in cyanide dies earlier from lack of oxygen than a normal control animal.

5. Since cyanides decrease both oxygen consumption and CO_2 production in *Planaria*, the increase by cyanide in susceptibility to lack of oxygen can mean only that cyanide and lack of oxygen are to some extent additive in their action on living protoplasm, i.e., their action must be in certain respects identical or similar in character.

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